

UNIVERSITÀ POLITECNICA DELLE MARCHE Repository ISTITUZIONALE

Improved nutritional quality in fruit tree species through traditional and biotechnological approaches

This is the peer reviewd version of the followng article:

Original

Improved nutritional quality in fruit tree species through traditional and biotechnological approaches / Sabbadini, Silvia; Capocasa, Franco; Battino, Maurizio; Mazzoni, Luca; Mezzetti, Bruno. - In: TRENDS IN FOOD SCIENCE & TECHNOLOGY. - ISSN 0924-2244. - 117:(2021), pp. 125-138. [10.1016/j.tifs.2021.01.083]

Availability:

This version is available at: 11566/288046 since: 2024-04-05T16:01:33Z

Publisher:

Published DOI:10.1016/j.tifs.2021.01.083

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions. This item was downloaded from IRIS Università Politecnica delle Marche (https://iris.univpm.it). When citing, please refer to the published version.

IMPROVED NUTRITIONAL QUALITY IN FRUIT TREE SPECIES THROUGH TRADITIONAL AND **BIOTECHNOLOGICAL APPROACHES**

- Silvia Sabbadini^a, Franco Capocasa^a, Maurizio Battino^{bc}, Luca Mazzoni^{a**}, Bruno Mezzetti^{a*}
- ^a Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Ancona, 60131, Italy
- ^b Department of Clinical Sciences, Marche Polytechnic University, Ancona, 60131, Italy
- ^c International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang 19 212013, China
- ^{*}Corresponding author.
- **Corresponding author.
- E-mail addresses: 1.mazzoni@staff.univpm.it (L. Mazzoni), b.mezzetti@staff.univpm.it (B. Mezzetti)

Abstract

Background: The increasing evidences on the relation between diet and human health are driving the consumers toward the choice of foods with high amount of nutrients, considered the molecules responsible for the regulation of several metabolic and biochemical processes, and for the prevention of many chronic diseases. Among food products, fruits represent a natural source of many different nutrients and possess much importance in a balanced diet. The increase of the micro/macronutrient levels and the decrease of antinutrients amount are considered main objectives for the improvement of fruit trees nutritional value.

Scope and approach: This review summarizes many of the attempts made to increase the nutritional quality of fruit tree species during the last decades through the application of traditional and molecular breeding technologies, and the most recent New breeding techniques (NBTs).

Key findings and conclusions: New genotypes with improved nutritional quality have been obtained for different fruit tree species, either by increasing the content of well-known beneficial molecules, such as phenolic compounds, vitamins, and carotenoids, or by decreasing the levels of specific antinutrients. The development of molecular techniques and biotechnological tools allowed the identification and validation of candidate genes involved in the regulation of specific classes of nutritional compounds in fruit trees, and useful to obtain nutritionally improved products. NBTs represent alternative tools to classical breeding techniques, to regulate one or more fruit trees key nutrients in a more quick and precise manner. However, genetic engineering approaches still present public and regulatory concerns that strongly limit their exploitation.

Keywords

- Biofortification, nutrients, classical breeding, Marker assisted selection, New breeding techniques

53 Background

54 In the modern society consumers are becoming increasingly aware of the relationship between food 55 consumption and health benefits. An increasing number of evidences are indicating that several food 56 components can exert a positive effect on the physiological processes during lifespan. Foods with a high 57 content of bioactive compounds have been demonstrated to have a central role in chronic disease prevention 58 by exerting specific biological activities. Plant-based products represent the most consumed food worldwide, 59 irrespective of culture, location, and economic situation. Therefore, the development of new plant-based food 60 products with improved nutritional properties, through conventional and/or molecular breeding techniques, could have a great impact on the improvement of population health (Mezzetti, 2013; Pott et al., 2019). 61

62 Plant components of dietary interest are called nutrients and are responsible for the regulation of metabolic 63 and biochemical processes, the repair of body tissues, growth, and energy production. Plant nutrients can be 64 classified into three categories: macronutrients (carbohydrates, lipids, proteins and fibers), micronutrients 65 (vitamins, minerals and secondary metabolites), antinutrients (molecules that negatively influence the 66 bioavailability of macro and micronutrients) (Newell-McGloughlin, 2008) (Fig. 1). The increase of the 67 micro/macronutrients and the decrease of antinutrients levels are considered main objectives of many 68 breeding programs for the improvement of plant nutritional value.

69 Among the plant-based products, fruits, besides their attractive colour, taste and aroma, represent a natural 70 source of many different nutrients and possess much importance in a balanced diet. They usually contain no 71 cholesterol, high amount of water, and low calories associated with the absence of lipids (except for some 72 species, like avocado and olive). Among macronutrients, fruits contain good quantities of carbohydrates, in 73 particular, fructose, which is fermented in the large intestine where it forms small-chain fatty acids useful in 74 the treatment of children constipation. Among micronutrients, vitamins, such as A, B, C and E, represent one 75 of the main healthy compounds, given the high number of positive nutritional properties associated with their 76 consumption (Kaur et al., 2020a). Fruits are also rich in secondary metabolites (e.g. polyphenols, carotenoids, 77 and tocopherols), which possess many healthy properties, including anti-inflammatory and anti-tumorigenic 78 effects. In addition, fruits are characterized by antioxidant activities that significantly contribute to quench 79 the so-called reactive oxygen species (ROS), protecting lipids, proteins and nucleic acids from oxidation, and 80 reducing the insurgence of several related diseases and disorders (Kaur et al., 2020a). Therefore, it is evident 81 that improving the amount of micro and macronutrients in fruit species, as well as reducing the content of 82 undesired compounds, could bring many health benefits to the consumer, also with a lower consumption of 83 dietary fruits.

84 Currently, different strategies have been applied to modify the content of bioactive compounds in foods. The term "biofortification" or "biological fortification" describes a food crop with enhanced nutritional 85 86 compounds developed through different tools, like agronomic practices (i.e fertilization, plant growth-87 promoting soil microorganisms), conventional breeding, or biotechnological techniques (Garg et al., 2018). The most accepted method for biofortification is conventional breeding, being a more sustainable and cost-88 89 effective system compared to those other two approaches. Many improved varieties have been developed 90 through inter-specific hybridization, using wild species to expand the breeding pool available for the 91 introgression of the characters of interest during the first stages of crossing (Karanjalker & Begane, 2016). 92 Until now, many attempts for the improvement of nutritional quality in staple crops have been made, while 93 little efforts have been done in fruit tree species. In fact, most of the breeding programs are mainly focused 94 on agronomical and commercial traits, like plant yield and architecture, earliness of production, and 95 resistance to biotic and abiotic stresses (Karanjalker & Begane, 2016). There are many reasons why 96 researches aimed at improving the nutritional quality of fruit trees have been limited. Fruit qualitative 97 characteristics are usually quantitatively inherited, and the basic knowledge about the metabolism of some 98 compounds of interest is still lacking, due to the fact that it comprises complex interactions among thousands 99 of metabolic pathways. Furthermore, many fruit tree species are characterized by a long juvenile phase that 100 makes breeding process particularly long (e.g. avocado tree can take up to 15 years before flowering, and a recent breeding program on apple took 26 years to release three new commercial varieties) (Migicovsky & 101 102 Myles, 2017). After this long period, there is the possibility to obtain a progeny that does not express the targeted traits, and consequently, to produce new selections not of interest for the breeders. In addition, when 103 we consider that other external factors such as natural calamities, environmental pressure and pathogens 104 105 attack could lead to a loss of yield or, in the worst case, of new individuals, it is clear that the breeding of 106 fruit trees for quality improvement is particularly complicated. For all these reasons, the research has been 107 stimulated to find new methodologies and technologies able to amplify the starting gene pool variability, to

shorten the breeding processes and facilitate the combination of traits of interest in the new progeny(Karanjalker & Begane, 2016).

110 In particular, marker-assisted selection (MAS) has emerged as a very promising strategy for improving the

breeding selection processes, especially since the complete genome of various fruit species has been made available. This molecular tool can dramatically shorten the number of crossing cycles required to remove the

113 unwanted genes in the backcrossing program.

114 In order to use wild relatives for MAS, the first step is to discover markers for the traits of interest. Linkage

- 115 mapping (LM) and genome-wide association studies (GWAS) are methods used to analyse the genotype-
- 116 phenotype relationships (Migicovsky & Myles, 2017). Selection by molecular markers is particularly useful
- 117 when fruit characteristics are difficult or expensive to recognize or are visible only after a long juvenile 118 period (qualitative traits) (Karanjalker & Begane, 2016). In addition, MAS can decrease the costs related to
- 110 period (quantative traits) (Naranjaiker & Begane, 2010). In addition, MAS can decrease the costs related to 119 the breeding of some perennial plants up to 43% during the first 6-8 years, compared to a traditional breeding
- 120 program (Edge-Garza et al., 2015).
- Despite the application of these efficient genomic-based techniques, traditional breeding still presents some limitations, which arise mainly from the procedure followed to obtain a genetically improved plant. Several breeding generations are still necessary to introgress the trait of interest in a local elite cultivar, from a minimum of seven for clonally propagated crops like banana, up to 17 for cross-fertilizing crops like rice (Shimelis & Laing, 2012). In addition, other bottlenecks related to traditional breeding techniques are represented by linkage drag issues and by the limited gene pool where to source the sequences of interest, which is constrained to sexually compatible plants (De Steur et al., 2015; Kanchiswamy et al., 2015).
- 128 In terms of biofortification, alternative agronomic tools to traditional and molecular breeding are now 129 available, which are represented by genetic engineering approaches including the New breeding techniques 130 (NBTs) (cisgenesis/intragenesis, RNAi, genome editing). These strategies allow the introduction of one or 131 more traits able to improve the biosynthesis, accumulation and/or bioavailability of health-promoting 132 compounds, or the knockdown/knockout of specific genes responsible for the accumulation of undesirable 133 molecules (Uncu et al., 2013). In addition, the accumulation of one or more key nutrients can be targeted to specific edible organs or redistributed between tissues through genetic engineering techniques, which 134 135 represents an advantage especially for those compounds which are mainly distributed in not edible portions 136 of a crop (Zhu et al., 2013). Till now, transgenic biofortified crops have been mainly characterized by the increase in specific micro/macro nutrients especially in staple crops, with the aim of mitigate the daily diet 137 deficiency of people from developing countries. The most famous example is Golden rice, a variety of rice 138 139 where provitamin-A pathway was genetically modified to produce ß-carotene in the endosperm of the grain, 140 with the aim of addressing vitamin A deficiency in at least 26 countries (Stokstad, 2019). However, this and
- many other promising transgenic biofortified crops with an effective benefit for the consumer are still facing
 several commercialization barriers mainly due to the regulatory issues and public concerns linked to
 genetically modified plants (GMPs) (De Steur et al., 2015).
- 144 The development and application of NBTs in agriculture should arise lesser biosafety concerns and increase 145 consumer's acceptance if compared with the first generations of GMPs, especially because some of these 146 techniques can be exploited to obtain transgene-free plants (Limera et al., 2017). This is the case of cisgenesis and intragenesis that consist in the introduction of sequences with regulatory regions isolated from 147 sexually compatible species or the plant itself, as done for traditional breeding, avoiding the addition of 148 foreign sequences in the host genome (Schouten et al., 2006). Nowadays, cisgenesis and intragenesis have 149 150 been applied to a limited group of plant species, especially for the lack of complete genomic information and knowledge on regulatory sequences for many crops. In addition, the identification of transformed events in 151 152 these kind of transformation systems must rely on the use of cisgenic/intragenic reporter genes, as well as cisgenic/intragenic promoters and selectable markers (Carvalho & Folta, 2017; Krens et al., 2015). 153
- 154 Nutritional improvement of plants through biotechnological tools can also be achieved by reducing the level 155 of antinutrients/toxins through the regulation of specific protein coding genes, for example by exploiting RNA interference (RNAi) (Katoch & Thakur, 2013). RNAi is a naturally conserved mechanism in plants. 156 157 that acts by preventing messenger RNA translation into protein through the action of double strand RNA (dsRNA) molecules which target the silencing of specific transcripts in a sequence-dependent manner 158 (Mezzetti et al., 2020). This technology has been applied for the biofortification of several plant species by 159 reducing the presence of undesired molecules from edible tissues without affecting the level of these 160 161 compounds in other parts of the plant. For example, cottonseed, which is considered an excellent resource of edible oilseed and a high-quality protein tissue, has been engineered through RNAi methodology to reduce 162 163 its gossypol content, thus allowing its use for food or as feed, otherwise limited by the presence of this toxic

terpenoid (Rathore et al., 2020). Another way to exploit gene silencing to increase specific bioactive
compounds consists in the silencing of specific key-enzyme encoding genes within a metabolic pathway,
which are known to negatively regulate one or more biosynthetic fluxes (Zheng et al., 2020).

167 The so called "block" strategy mediated by RNAi mechanism, that acts by downregulating up- or 168 downstream enzymes activities limiting specific metabolic flux, can also potentially be obtained through the use of genome editing tools. They have recently been enriched by the type II clustered regularly interspaced 169 short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) system, adapted from the natural 170 171 immune system against viruses and plasmids of the Streptococcus pyogenes (Zheng et al., 2020). This system can lead to single-point mutations, integration or deletions of target genes through the action of an 172 173 engineered Cas9 protein that can be reprogrammed and guided by a synthetic guide RNA to cleave specific target DNA sequences. The repair step subsequent to the double strand break induced by the two Cas9 174 175 nuclear domains can be exploited to delate, change or insert a single nucleotide in a target gene or even add a 176 whole new sequence (Kaur et al., 2020a; Limera et al., 2017).

177 All these biotechnological tools can be applied to fruit tree species for many purposes, although the introduction of one or more genes encoding for specific traits, or the expression of RNAi gene constructs, as 178 179 well as of engineered CRISPR/Cas9 systems, are frequently limited by the availability of efficient in vitro regeneration and transformation systems. These protocols are often genotype-dependent and are 180 181 recommended to be developed from somatic tissues to maintain the genetic uniformity of target clonally propagated plants, like fruit tree species (Ricci et al., 2020; Sabbadini et al., 2019). However, several studies 182 183 have been carried out during the last decades for the genetic improvement of fruit trees, and NBTs represent 184 an additional tool to be exploited by plant breeders also for the nutritional enhancement of these species 185 (Kanchiswamy et al., 2015).

186 This review will provide an overview of the more recent studies performed on fruit tree species to increase 187 their micro/macro nutrient content (phenolic compounds, vitamins, minerals, carotenoids, carbohydrates and 188 lipids), as well as for reducing antinutritional compounds through both traditional breeding techniques or by 189 the application of genetic engineering strategies, including the more recent NBTs.

191 Phenolic compounds

190

192 Phenolic compounds are a large group of plant molecules with function in tissues pigmentation, and in the 193 defence against biotic and abiotic stresses, thanks to their strong antioxidant activity, which also allows the 194 protection of human body from oxidative damages when they are assumed through the diet (Pott et al., 2019). 195 Phenolic compounds have been the most studied secondary metabolites in conventional fruit tree breeding programs as parameter of nutritional quality. A combination of conventional and non-conventional 196 197 techniques has been used for such studies including inter- and intraspecific crossing, implementation of 198 various -omics technologies and the application of genetic engineering strategies. Plenty of studies are available in literature describing the attempts that have been made to obtain new genotypes with improved 199 200 phenolics amount, usually exploiting the availability of wild germplasm. Wild genotypes are believed to possess strong antioxidant activity, higher than the cultivated ones, but also among different wild accessions 201 202 there could be a difference in antioxidant capacity and phenolic content. In many fruit tree species the use of 203 wild accessions to produce genetic diversity and to increase the amount of phenolics in commercial genotypes has become a common strategy, as in persimmon (Ramírez-Briones et al., 2019), papaya (Iamjud 204 et al., 2016), boysenberry (Ryu et al., 2017), saskatoon berry (Lachowicz et al., 2017), blueberry (Yousef et 205 al., 2016), strawberry (Mazzoni et al., 2020), apple (Wang et al., 2018), olive (Pérez et al., 2019), 206 207 blackcurrant and redcurrant (Stanys et al., 2019).

208 Among phenolic compounds, flavonoids are a major group of bioactive agents which comprises more than 9000 bioactive molecules, included chalcones, flavones, flavonols, flavanones, isoflavonoids and 209 210 anthocyanins, synthetized through the phenylpropanoid pathway (Zhu et al., 2013). In particular, 211 anthocyanins have been associated to a series of potential benefits for human health, included the prevention 212 against certain types of cancer, inflammation, and cardiovascular diseases (Mazzoni et al., 2019). For this reason, these secondary metabolites have been the subject of many studies in which the regulation of their 213 214 biosynthesis-related genes was tempted, acting mainly at the level of their transcription factors, which 215 include MYB complex, basic helix-loop-helix (bHLH), and WD-repeat proteins (Peng et al., 2020).

MYB genes have been identified in several fruit tree species, with functions in plant development control and
 regulation of different bioactive compounds (Allan & Espley, 2018). Most MYB transcription factors
 positively modulate genes involved in flavonoid biosynthetic pathway in several fruit tree species (Table 1).
 In particular, the apple *MdMYB10* gene, an allele of *MdMYB1/MYBA*, determines the red pigmentation of the

Type 1 (red fruit flesh, skin, leaves, and other vegetative tissues) apple. In Type 2 (only red flesh) apples,
another MYB transcription factor close to MdMYB10, namely MdMYB110a, is responsible for the red
pigmentation of flesh (Wang et al., 2018).

223 Great progress has been made in the application of molecular techniques for the genetic improvement of 224 Prunus spp., especially since the high-quality peach genomic sequence has been made publicly available since 2010 (Aranzana et al., 2019). In sweet cherry, skin and flesh colour is related to anthocyanin 225 production, and the major responsible QTL was mapped on linkage group (LG)3 to an interval containing the 226 227 *PavMYB10* gene. LG3 was also shown to have major genes/QTLs controlling the anther colour in almond \times 228 peach progenies and the skin colour in peach, Japanese plum, and apricot (Aranzana et al., 2019; García-229 Gómez et al., 2019). 14 QTLs were recently successfully mapped in peach, among them, four QTL clusters were identified on four different LGs with different traits: a QTL for flavonoids accumulation was mapped 230 231 on LG7 together with the OTL associated to sucrose synthesis. Furthermore, a major OTL for antioxidant 232 capacity, flavonoids and anthocyanins was detected on LG5, indicating the presence of genes involved in the 233 accumulation of nutritional compounds in this region. The candidate genes present in this phytochemical QTL cluster, mapped on LG5, were identified and are the Prupe.5G105700, Prupe.5G105800 (both 234 235 encoding for MYB-related transcription factors involved in the flavonoid metabolism), and Prupe.5G116700 236 [annotated as anthocyanidin reductase ((2S)-flavan-3-ol-forming)], an important regulator of flavonoid biosynthesis in plants (Abdelghafar et al., 2020). 237

Given their high potential for polyphenols content, small fruits called "berries" received particular attention
in the development of new genomic-based techniques (Table 1). A metabolomic approach has been applied
to identify bioactive compounds in the raspberry population deriving from the crossing of 'Latham' x 'Glen

Moy'. The eight main anthocyanins were all mapped on LG1, irrespective of year and type of cultivation, and the candidate genes encoding for bHLH, NAM/CUC2 like protein, and bZIP transcription factors were

and the candidate genes encoding for bHLH, NAM/CUC2 like protein, and bZIP transcription factors were
identified. On the same population, QTLs associated with phenolic pool were detected on LG2, LG3, LG5,
and LG6 (Mccallum et al., 2018).

245 The mapping of a wild strawberry F. vesca collection allowed the identification of 100 metabolic (m) QTLs for 24 different nutritional compounds. Total anthocyanins were mapped at three different one-year mQTLs 246 247 (on LG3, LG4, and LG5), while the under-accumulation of the most abundant anthocyanin (pelargonidin-3-248 glucoside) was mainly linked to a major mQTL on LG5, and the major QTL on LG2 was linked to the over-249 accumulation of the cyanidin-3-glucoside. Some major mOTLs were also detected for single flavanols 250 (kaempferol on LG1 and LG7, and guercetin on LG2). Stable mOTLs for total and specific flavan-3-ols were 251 mapped on region LG5, while positive major stable QTLs for hydroxycinnamic acid derivatives were mapped on regions LG2 and LG4. Two positive stable QTLs for ellagic acid accumulation were mapped on 252 253 LG1 and LG4 regions (Urrutia et al., 2015). Hossain et al. (2018) identified, in addition to the well-254 characterized positive regulator FaMYB10 gene, other putative key genes involved in anthocyanins 255 biosynthesis in Fragaria x ananassa (Table 1).

256 The identification of candidate genes of interest through integrative metabolomics and transcriptomics 257 approaches represents a powerful strategy also for the application of biotechnological tools. The modulation 258 of flavonoid biosynthetic pathway through genetic engineering techniques has been shown to be an efficient 259 alternative strategy to obtain fruits with enhanced nutritional level, and improved health effects (Lin-Wang et 260 al., 2014). As mentioned before, MdMYB10 transcription factor is responsible for anthocyanin accumulation during apple fruit development; this was also confirmed by a research study where the MdMYB10 gene was 261 262 overexpressed in apple plants leading to a significant increase in anthocyanin content correlated to a highly pigmented phenotype in the transformed lines (Espley et al., 2007). These genetically modified apples were 263 264 used in mouse feeding trials, and the results obtained showed that a dietary flavonoid consisting of MYB 265 apples led to a significant decrease in some inflammation markers and modulation of gut microbioma 266 (Espley et al., 2014). The MdMYB10 gene was also used to develop an A. tumefacien-mediated 267 transformation system for the production of cisgenic apple plants, that were visually identified through the red coloration conferred by anthocyanins accumulation, avoiding the use of transgenic selectable marker 268 269 genes (i.e antibiotic or herbicide resistance genes), which are often exploited in transgenic vectors to give a 270 selective advantage only to the plant cells that have integrated the sequence of interest (Krens et al., 2015). Recently, the involvement of the MdWRKY11 gene in the accumulation of anthocyanins after its 271 272 overexpression in red-fleshed apple has also been suggested, which significantly increased the expression of 273 some MYB transcription factors as well as of structural genes (Liu et al., 2019). The MYB10 genes from 274 Fragaria vesca (FvMYB10) and from the cultivated strawberry Fragaria x ananassa (FaMYB10) were also 275 overexpressed in these two species respectively, producing F. vesca fruits with purple skin and red flesh, and

an increase in anthocyanin concentration in different plant tissues of both these transgenic species (Kui et al.,
2014; Lin-Wang et al., 2014). Among MYB family, repressor of anthocyanin and proanthocyanidin pathway
genes have also been characterized in different fruit tree species (Table 1). For example, when the *FaMYB1*gene was overexpressed in strawberry fruit or silenced by an RNAi-construct, a decrease or an accumulation
in anthocyanin content were observed in *F. x ananassa*, respectively (Kadomura-Ishikawa et al., 2015).

As mentioned above, transcription factors other than MYB, among the ternary MYB-bHLH-WD40 (MBW) complex, have been individuated as positive or negative anthocyanin regulators. In particular, bHLH proteins have been characterized as necessary in flavonoid biosynthesis in different fruit tree species, like apple, kiwi, and grapevine (Allan & Espley, 2018; Wang et al., 2019). As an example, transgenic apple cv. Gala lines were obtained overexpressing the *MdbHLH3* transcription factor, which induced an increase in anthocyanin production in shoots and roots, and a higher transcript level of both the *MdDFR* and *MdUFGT* genes (promoters of the anthocyanin biosynthesis genes) in apple skin was observed (Xie et al., 2012).

Other candidate genes have been validated through genetic engineering techniques to improve anthocyanins content in fruit trees. In particular, the overexpression in the octoploid strawberry of the *ANS* gene, which catalyses the synthesis of anthocyanidins, induced an increase in total antioxidant capacity, and higher concentrations of phenolics and flavonoids were observed. The fruit extract collected from the transgenic lines obtained showed a cytotoxic effect on hepatocellular carcinoma (Giampieri et al., 2018).

Recently, also the *Reduced Anthocyanins in Petioles (RAP)* gene was characterized in the cultivated strawberry through both overexpression and CRISPR/Cas9-mediated knockout strategies, that determined its role in fruit coloration and anthocyanin accumulation at anthesis (Gao et al., 2020).

296 In addition to flavonoids, stilbenes are another group of plant-derived phenolic compounds, synthetized via 297 the phenylpropanoid pathway, which are known for their health benefits and role in plant defence against pathogens and pests (Kiselev & Dubrovina, 2020). One of the most studied stilbenes is resveratrol, a 298 299 naturally occurring polyphenol in several plant species like grapevine and blueberries, which has gained particular interest due to its antioxidant, antiangiogenic, and anticancer activities (Kiselev & Dubrovina, 300 301 2020). One of the key enzymes involved in the stilbene biosynthetic pathway is the stilbene synthase (STS), which has been the focus of several biotechnological approaches to increment the concentration of this class 302 303 of secondary metabolites in plants, especially in grapevine (Kiselev & Dubrovina, 2020). Some attempts 304 have also been made in other fruit tree species, such as kiwifruit overexpressing the stilbene synthase gene 305 (Vst1) isolated from different Vitis spp., which led to an increase in piceid (resveratrol-glucoside) content 306 (182 µg/g fresh weight in young leaves) in the transgenic lines (Kobayashi et al., 2000). Similar results were 307 observed in genetically modified apple expressing the Vst1 gene, which led to the accumulation of 308 resveratrol derivatives in fruits (Rühmann et al., 2006).

Some biotechnological approaches related to phenolic compounds were also focused on the use of RNAi mechanism for both validating the function of genes of interest, and to redirect specific biosynthetic pathways. In strawberry the downregulation of the *anthocyanidin reductase* (*ANR*) gene shunted the proanthocyanidin pathway to a premature and ectopic anthocyanin biosynthesis (Fischer et al., 2014).

314 Vitamins

313

315 Vitamins are fat-soluble (A, D, E and K) or water-soluble (B complex and C) micronutrients, with different 316 roles in plant metabolism and essential to human health and survival, indeed the human body cannot 317 synthetize these compounds *de novo*, or it can only in insufficient quantities. Vitamins need to be absorbed 318 through different foods and nutritional supplements to fulfil their diverse physiological functions and prevent 319 clinical deficiencies (Fitzpatrick & Chapman, 2020).

320 Vitamin E (comprising tocopherols and tocotrienols) has been proved to have cholesterol lowering effects (Arya et al., 2019), and its antioxidant and radical scavenging activity has been extensively proved (Fritsche 321 322 et al., 2017). A sufficient uptake of vitamin E helps to prevent chronic diseases (as cancer, cataracts, and 323 atherosclerosis) and neurological disorders (in particular in elderly people), as well as to reduce infertility (Fritsche et al., 2017; Strobbe et al., 2018). Currently, plenty of QTLs and association mapping studies 324 related to vitamin E have been performed in major crops including barley, maize, rapeseed, rice, soybean, 325 326 and tomato (Fritsche et al., 2017), but little is known about their gene regulation in fruit trees. In avocado, a metabolomic study on Mexican accessions revealed a higher amount of this compound compared to the 327 328 control cultivar, suggesting the VTE3 and VTE4 genes implied in tocopherols biosynthesis (Peraza-329 Magallanes et al., 2017). For oil crops, the enhancement of vitamin E in fruits is important in order to 330 prevent oil peroxidation and thus to improve oil quality. In fact, as demonstrated for virgin olive oil, the 331 amount of tocopherol in the final product depends on the quality of the olive fruit, which in turn can change

according to the genotype (Table 1). With a single crossing generation, it is possible to obtain new genetic
variability for the improvement of this compound (Pérez et al., 2019). The genetic variability associated to
the total content of vitamin E has also been studied in Turkish and Afghan almond genotypes, identifying
some interesting cultivars for future breeding programs aimed at increasing vitamin E (Çelik et al., 2019;
Zamany et al., 2017).

Vitamin A is important for many functions in human body, for example its deficiency is related to 337 338 xerophthalmia, a pathophysiological condition that starts with night blindness, and ultimately leads to 339 complete blindness. Vitamin A is also related to the functionality of immune system and its deficiency could 340 lead to a higher child mortality for severe infections as malaria, diarrhoea and measles. Vitamin A deficiency 341 also contributes to the insurgence of anaemia, being involved in the iron metabolism; furthermore, this 342 vitamin is important in the growth process, being necessary in spermatogenesis as well as for proper embryo 343 growth (Strobbe et al., 2018). The geographical and endemic location of vitamin A malnutrition clusters are 344 often characterized by the presence of infectious diseases, poverty, and poor infrastructure that do not assure 345 availability of foods rich in vitamin A. As bananas are key staples in those geographical areas (Africa and 346 South-East Asia), biofortification efforts for vitamin A content in these fruits have been made to ameliorate 347 the nutritional status of these vulnerable populations. The most common conventional breeding strategy for 348 banana is to cross seed-fertile 3x cultivars to 2x accessions that carry the candidate genes, then to select 4x 349 and 2x hybrids from intermediate products, and to cross these hybrids to obtain sterile 3x hybrids with the character of interest. The recent release of the draft genome sequence of Musa spp. has allowed genomic 350 351 studies aimed at identifying candidate genes for nutritional and agronomic traits of interest, and for the 352 optimization of molecular techniques aimed to speed up banana breeding (Amah et al., 2019a).

353 Vitamin C or ascorbic acid is one of the most powerful antioxidant vitamins and acts as important ROS 354 scavenger. Besides antioxidant, it exerts plenty of functions in the human body for maintaining a good health. 355 In fact, it is involved in many metabolic and energetic processes, it reduces the cholesterol levels by 356 protecting against atherosclerosis, as well as some types of infections, and can counteract some tumours 357 development. A deficiency of ascorbic acid could lead to anaemia or to the insurgence of scurvy, a disease characterized by neuropathy, spontaneous bleeding, haemolysis, oedema, jaundice, and eventually death 358 359 (Strobbe et al., 2018). It is known that, among foods, fruits are the best source of this vitamin, and for this 360 reason many studies have been done to explore the genetic variability of different species to identify genotypes suitable for breeding programs aimed at increasing the amount of vitamin C in target cultivars 361 (Surva et al., 2018). These kind of screening were conducted on *Rubus* spp. (Surva et al., 2018), on papaya 362 (Iamjud et al., 2016), on strawberry (Zhong et al., 2017), and on apple (Fang et al., 2017). In this latter study, 363 ascorbic acid concentration in 457 apple accessions was negatively correlated with the expression of three 364 365 regulatory genes, namely MdGGP1, MdDHAR3-3, and MdNAT7-2. In Actinidia spp., the sequencing of a 366 hybrid genotype showed the presence of a QTL on chromosome 26 denoted as qAsA26.1, and the region harbouring this QTL could be considered a supergene for vitamin C determination (McCallum et al., 2019). 367

368 Vitamins level enhancement in plants through genetic engineering techniques represents a valid alternative, that has been limited to a few crops, like Arabidopsis and rice, that do not produce edible fruits, or tomato, 369 370 that is considered the model of fleshy fruits (Fenech et al., 2019). The overexpression of the strawberry Dgalacturonic acid reductase (FaGalUR) gene in tomato plants led to an increase in L-ascorbic acid also 371 associated to an enhanced tolerance to different abiotic stresses or to complex changes in metabolites 372 (Fenech et al., 2019). Another example of fruit tree-derived gene ectopic expression in model plant is 373 374 represented by the apple homogentisate phytyltransferase gene (MdHPTI), which was overexpressed in 375 tomato leading to an increase of tocopherol up to 3.6-fold and 1.7-fold in transgenic leaves and fruits, 376 respectively (Seo et al., 2011). These studies represent some examples of effective strategies to validate 377 candidate genes in model plants useful to obtain economically important fruit trees nutraceutically improved. 378 One of the few examples of fruit tree engineering for vitamins enhancement is represented by transgenic 379 strawberry with increased vitamin C content. In this study, seven independent lines were obtained showing a 380 two-fold increase in ascorbate concentration through the overexpression of the kiwifruit GDP-galactose 381 phosphorylase gene (GGP) (Bulley et al., 2012).

383 Minerals

382

384 Unlike vitamins, plants cannot synthetize minerals, but they take up mobile ions from the soil through 385 specific transporters directly into the root plasma membrane, or they synthetize chelating agents able to 386 mobilize minerals absorbed from the soil (Zhu et al., 2013). Human body needs several metals in small 387 amounts, which are used for example as cofactors for several enzymatic reactions or transcription factors. Furthermore, some minerals are of particular interest in the prevention of some diseases. Selenium, for example, is considered protective against certain types of cancer, and its deficiency has been related to pathogenesis of a virus involved in the Keshan's disease. Good levels of zinc in the body have also been associated to normal growth and reproduction, indeed, this compound is involved in the basic functions of the cell (Schneeman, 2001).

At our knowledge, no studies have ever been carried out to ameliorate the content of minerals in fruit species 393 394 through conventional breeding. Only a few comparative studies have been performed to evaluate the mineral 395 content in different genotypes, as in peach, but no concrete research has been carried out aimed at improving 396 breeding programs (Bobis et al., 2017). Most of the studies describing transformed crops with enhanced 397 minerals bioavailability and content through genetic engineering techniques have been carried out on staple crops. Iron-deficiency anaemia is one of the most prevalent micronutrient deficiencies in the world, and iron 398 399 increase by transformation of staple crops like rice, wheat or grains showed some disadvantages due to the 400 high level of phytic acid (inhibitor of iron adsorption) present in these species, thus alternative target crops should represent a better choice (Kumar et al., 2011). To our knowledge a unique example exists of mineral 401 fortification in fruit tree species, represented by the transgenic banana with a higher level, compared to the 402 403 wild type control, of both iron and zinc through the overexpression of the soybean ferritin. The results presented in this study showed a 6.32-fold increase in iron accumulation, and a 4.58-fold increase in zinc 404 405 level detected in the leaves of the transgenic lines. Although no data have been published yet on the increase 406 of both these minerals also in the transgenic fruits of fortified banana, this study showed the possibility of 407 developing functional fruit tree species helping to overcome iron and zinc deficiencies (Kumar et al., 2011). 408

409 Terpenes

410 Terpenes is one of the largest class of compounds in fruits and vegetables; among them, the carotenoids 411 family, consisting of carotenes and xantophylls, is the wider group with more than 600 naturally occurring compounds. In plants, these lipophilic isoprenoids pigments play vital function in the photosynthesis 412 413 apparatus, in addition to their role as hormones precursors and photooxidative protectors (Zheng et al., 2020). Of all these compounds only a few dozen are common in the human diet, playing essential roles as 414 415 antioxidant compounds and ROS scavengers, useful to prevent both cardiovascular diseases and some types 416 of cancer, but mainly for being essential dietary precursors of vitamin A (provitamin A). In mammals, 417 provitamin A carotenoids are converted into retinol and other related retinoids that exert an important function in gene regulation and for the vision (Zheng et al., 2020). Recent studies have also suggested a 418 protective role of these compounds against macular degeneration, cognitive function impairment, type-2 419 420 diabetes, and obesity (Rodriguez-Concepcion et al., 2018).

421 One of the most studied fruit species for its carotenoid content is banana, considered one of the top 10 crops 422 by production, and the main staple food in some countries. Large-scale germplasm studies have been set up on this species to identify genotypes with high levels of provitamin A (Amah et al., 2019b), leading to the 423 release of some new cultivars (Apantu, Bira, Pelipita, Lai, and To'o) in Eastern Democratic Republic of 424 425 Congo and Burundi (Garg et al., 2018). Other species have also been screened for carotenoid content in new breeding lines, as papaya (Iamjud et al., 2016), and strawberry, where eQTLs were discovered for published 426 427 alleles of the strawberry phytoene synthase (FanPSY) and z-carotene desaturase (FanZDS) genes (Barbey et al., 2020). Citrus spp. are among the carotenoid-richest fruit species, so the study of this metabolism in 428 Citrus spp. has received particular attention (Table 1). A F1 population from a Citrus reticulata intraspecific 429 cross indicated that the carotenoids variation was determined by 17 OTLs on chromosomes 2, 3, 5, 6, and 9. 430 In particular, the QTL on chromosome 2 accounted for more than 20% of the variation of total carotenoid 431 432 content (Zheng et al., 2019). In three pummelo cultivars, a metabolomic study revealed the involvement of 12 differentially expressed genes in carotenoids biosynthesis (Jiang et al., 2019). The carotenoid biosynthetic 433 434 pathway has also recently been studied in papaya, finding six genes related to carotenoids biosynthesis 435 (Table 1), regulated by the transcription factors CpbHLH1/2 (Zhou et al., 2019). Finally, a recent study on grapevine helped in understanding the carotenoids biosynthesis in this species, indicating that the 436 downregulation of the VvDXS, VvDXR, VvGGPPS, VvPSY, and VvCCD genes might also be responsible for 437 the decrease of carotenoids amount (Leng et al., 2017). This knowledge gives an important genetic 438 439 foundation for future MAS into carotenoid fruit tree breeding programs.

440 Metabolic engineering studies have enhanced carotenoids content in different plant species through some
441 main common strategies, which were focused on "pushing" the metabolic flux upstream of a target
442 compound, generally β-carotene, or by "blocking" the expression of genes downstream of a desired
443 compound, and downregulating enzymes responsible for the degradation of carotenoids or competing for the

444 same substrate (Watkins & Pogson, 2020). One of the most popular approaches for the enhancement of 445 carotenoids production has been the overexpression of the phytoene synthase gene (PSY/CRTB) to obtain higher level of β-carotene (the precursor of pro-vitamin A) in the final target plants (Table 1). As already 446 mentioned, one of the most famous examples of food biofortification through genetic engineering are Golden 447 448 rice and Golden rice 2, presenting a total level of β-carotene up to 31 µg/g (Paul et al., 2017). A similar strategy has also been applied to obtain the Golden banana. The genetic improvement of domesticated 449 450 bananas by conventional breeding is hampered by the very low male and female fertility, thus genetic 451 engineering becomes a valid alternative tool. Pro-vitamin A biofortified Cavendish banana has been obtain by overexpressing the *phytoene synthase 2a* (*PSY2a*) gene derived from the Fe'i banana, which is naturally 452 high in ß-carotene. One of the transgenic lines obtained reached 55 µg/g dry weight (dw) of ß-carotene 453 equivalent (β -CE), compared to the wild-type control with a content between 1 and 4 μ g/g dw β -CE (Paul et 454 455 al., 2017).

- 456 The overexpression of PSY gene has not always been a successful strategy for the modulation of carotenoid content in plant, indeed some researchers have underlined the induction of off-target effects as a 457 consequence of PSY ectopic expression. An example is the transgenic tomato overexpressing the PSY gene 458 459 with an increased content of carotenoid, but with dwarf phenotype, which was attributed to a decrease in gibberellin A₁ synthesis, competing in the same metabolic flux (Watkins & Pogson, 2020). Recently, a 460 461 different gene other than *PSY* was ectopically expressed in apple to increase the level of different carotenoids. In particular, the overexpression of the *DXR* gene from *A. thaliana*, encoding for one of the key enzymes 462 463 regulating the plastidial isopropenoid pathway, led to an up to 3-fold increase in individual and total 464 carotenoids compared to the non-transgenic control (Arcos et al., 2020). Another important issue in terms of 465 carotenoid fruit biofortification, and in general for nutritional quality enhancement in plants, is the bioavailability of a specific compound, that in the case of carotenoids defines their digested fraction 466 467 available for human body physiological functions or storage. Some fruit tree species like papaya and citrus fruits are rich of esterified B-cryptoxanthin, which helps in the prevention of osteoporosis and certain kinds 468 469 of cancers, in addition to its provitamin A function. Although its structure characterized by a unique β -ring can lead to the production of only half the retinol molecules of ß-carotene, its esterification allows a higher 470 471 level of stability and bioavailability for this compound, thus making it another valuable target gene to 472 increase carotenoids in plants (Watkins & Pogson, 2020).
- 473 The so called "block" strategy, that exploits RNAi mechanism to downregulate genes that negatively impact 474 the accumulation of target carotenoids, has been used to obtain transgenic orange fruits with up to 36-fold ß-475 carotene content in the pulp. This achievement has been possible through the silencing of the β -carotene 476 hydroxylase ($Cs\beta$ -CHX) endogenous gene, which acts by converting β -carotene into xanthophylls (Pons et al., 477 2014). The recent discovery and development of the CRISPR/Cas9 biotechnological tool has allowed the 478 precise disruption of specific genes activity involved in carotenoid biosynthesis. For example, a ß-carotene-479 enriched Cavendish banana was obtained through CRISPR/Cas9-based approach targeting the lycopene 480 epsilon-cyclase gene, showing an increase in β -carotene up to 6-fold (about 24 µg/g) compared with the 481 unedited control (Kaur et al., 2020b). Similar studies have also been carried out on sweet orange and kiwifruit as proof of concept, by targeting their endogenous PDS gene, leading to a decrease in total 482 carotenoids; these and other recent studies have demonstrated the potential use of CRISPR/Cas9 tool for 483 precise gene knockout in several fruit tree species aimed to their nutritional enhancement (Kaur et al., 2020a). 484 485

486 Carbohydrates

487 Carbohydrates represent the main energy source in most people's diet and play an important role in energy 488 metabolism and homoeostasis. However, this contribution to energy metabolism could lead to weight gain when carbohydrates are consumed in excess (Mann et al., 2007). Consumption of carbohydrates, in 489 490 particular simple sugars, is commonly not recommended for subjects suffering of diabetes, particularly for 491 the postprandial increases in plasma glucose. Fruit consumption has the advantage to induce lower postprandial increases in plasma glucose when the main simple sugar is fructose. Another benefit from fruit 492 carbohydrates is their high amount in polymeric carbohydrates (e.g. starches and fructans); in particular, 493 fructans (oligosaccharides of fructose) are considered an important ingredient in functional foods because 494 they have been shown to promote a healthy colon while also reducing the incidence of colon cancer (Newell-495 496 McGloughlin, 2008). From a sensorial point of view, since the sweetness of fructose and sucrose are higher 497 than that of glucose and sorbitol, fruits containing higher levels of fructose and sucrose are desirable. Thus, the improvements or changes in carbohydrate content represent an important issue for fruits 498 499 quality/marketability and also for the total calories supplied in the human diet.

500 Sugars (soluble and insoluble) content in plants depends on different factors such as the genotype, ripening stage, and environmental conditions (Uncu et al., 2013). The amount of total carbohydrates and the 501 composition in simple sugars, have recently been investigated in different genotypes of several fruit tree 502 503 species, indicating which of them could be more suitable for consumption. A F1 population of apple 504 obtained from a crossing in China was useful to detect QTLs related to single sugars: QTL for glucose content was identified on LG4, while OTLs for sucrose, fructose, sorbitol and glucose were mapped on LG3 505 506 (Ma et al., 2015). Another study analysed 188 apple cultivars to evaluate the role of genes encoding SWEET 507 sugar transporters in fruit sugar accumulation. It was found that the MdSWEET15a and MdSWEET9b genes 508 were significantly associated with phenotypic variation in the sugar content of fruits (Zhen et al., 2018). In 509 sour cherry, different genotypes showed a different composition in both total carbohydrates and single sugars, with changing amounts of fructose, glucose and, at lesser extent, sorbitol (Proietti et al., 2019). Regarding 510 511 peach, a recent study on F2 population, deriving from a cross of two highly contrasting accessions, mapped 512 two suggestive QTLs for sugar content on LG3 and LG5. Interestingly, in this study a cluster of QTLs was 513 mapped on LG5 for phytochemical compounds and soluble sugars, underlying the high correlation between these traits, and assuming an important role of sugars in the regulation of phenolic biosynthesis (Abdelghafar 514 515 et al., 2020). In another study, major QTLs for sugar content in peach were also identified on LG4 and LG6 516 (Cirilli et al., 2016). Overall, these mapping trials suggest that peach sugar content is driven by several QTLs 517 with minor effects, often gathered in clusters, and that the hundreds of genes present within these regions 518 make it hard to detect the gene(s) linked to sugar content. Another Prunus species where significant QTLs 519 for soluble sugars content were identified is apricot, in particular, candidate genes involved in D-glucose and 520 D-mannose binding were located on LG4 (García-Gómez et al., 2019). Recently, a preliminary study on 521 papaya crossing population was performed to identify possible QTLs related to the fruit soluble sugar content, revealing the presence of a major QTL on LG7 for fruit sweetness (Nantawan et al., 2019). Sugar 522 523 content was also investigated in different genotypes of plum (Bobis et al., 2017), and boysenberry (Ryu et al., 524 2017).

In higher plants, the carbohydrates are mainly divided in soluble sugars and starch, and knowledge on this 525 portioning is a prerequisite to apply effective engineering strategies. An increase up to 8-fold in fructose 526 527 content at the expense of sucrose was observed in transgenic strawberry ripe berries when the fructose 6-528 phosphate 1-phosphotransferase (PFP) gene from Giardia lamblia was overexpressed (Basson et al., 2011). 529 In a recent work, the overexpression of the transcription factor *MdbHLH3* encoding gene in apple enhanced 530 the accumulation of carbohydrates in transgenic fruits by adjusting their allocation from sources to sinks (Yu 531 et al., 2020). In another work carried out on apple, an RNAi-construct was introduced to suppress the aldose-6-phosphate reductase gene encoding for a key enzyme for sorbitol synthesis. The transgenic lines obtained 532 533 showed a decrease in sorbitol concentration and much higher concentration of sucrose supply to fruits (Li et 534 al., 2018a). Sugars are transported for long distances in plants by a family of proteins that acts as sugar carriers, known as "sucrose transporters" (SUT). The pear sucrose transporter (PbSUT2) gene has recently 535 536 been characterized through its overexpression in tomato plants, which showed an increase in sucrose content 537 in the transgenic mature fruits compared to the non-transformed control, and a decrease in other sugars 538 concentration, like glucose and fructose (Wang et al., 2016a). 539

540 Lipids

541 Lipids have been recognized as one of the main sources of energy in the human diet, but their amount and 542 quality are fundamental for maintaining a healthy status. In fruits, fatty acids are the most common 543 compounds belonging to lipids, and they are mainly provided through oilseed crop plants. Fatty acids are 544 defined as organic compounds formed by an aliphatic chain and a carboxylic group normally bounded with glycerol-forming acylglycerides, considered necessary for all plant cells, essential for cell division and 545 546 survival, growth, and development. Saturated fatty acids have been associated to an increased risk of 547 cardiovascular diseases, as atherosclerosis, diabetes, and hypercholesterolemia. Contrarily, unsaturated fatty acids (UFA), as oleic, linoleic and linolenic acids, have been related to be preventive for cardiovascular 548 549 diseases insurgence, exerting beneficial effects on health (Assefa et al., 2017).

The difference in fatty acid patterns is a distinctive characteristic of cultivars within a species, as demonstrated in nuts, which are recognised as oil-rich fruits, but also in this case the content of fatty acids changes according to the species and the cultivar considered, and also on the basis of the geographical provenance. In particular, oleic acid is by far the most abundant fatty acid in hazelnut (about 80% of the total) followed by linoleic, palmitic and stearic acids, while walnut contains the highest concentrations of linoleic and linolenic fatty acids (up to 75% of the total). The study of different nuts genotypes provided information on new genetic sources for the development of new cultivars with promising quality characteristics (Kafkaset al., 2017).

558 Extra virgin olive oil is well known for its high amount of mono-unsaturated oleic acid and di-unsaturated 559 linoleic acid, which in turn derive from the fatty acid pattern of the olive fruit. Olive breeding programs are 560 currently running for the selection of new genotypes with improved agronomic and qualitative characteristics, including fatty acids content. The utilization of germplasm bank as source of genetic variability is a good 561 strategy for olive breeding. The analysis of fruit quality could predict the quality of the oil produced, as 562 563 several works reported a high correlation for fatty acids composition between the olive fruit and olive oil (De la Rosa et al., 2016). Several studies focused their attention on genes determining fruits and oils traits, and 564 the OeSAD2 gene was suggested to be the main contributor for oleic acid content in olive fruits, while the 565 OeFAD2-2 gene is responsible for the linoleic acid content (Hernández et al., 2017). Recently, genomic 566 567 studies on olive cultivars have been carried out to identify candidate OTLs related to the fatty acids content. 568 A single QTL has been identified on LG20 for oleic acid in the cultivar "Arbequina", and a QTL for linoleic 569 acid was identified in the same genomic position. The colocalization of mono- and di-unsaturated fatty acids in this location underlines the importance of this region in the fatty acid composition of olive oil, and the 570 571 OeSAD2 and OeFAD2-2 genes were confirmed to be candidate for oleic and linoleic acid content. QTLs for linolenic acid were detected on LG14 and LG19 for "Arbequina", on LG5 and LG15 for "Picual", and the 572 573 FAD7 gene resulted a good candidate to explain the QTL of linolenic acid (Hernández et al., 2017).

In olive, the stearoyl-acyl carrier protein desaturase (SAD) catalyses the first desaturation step leading to 574 575 oleic acid, and then to linoleic and linolenic acids through further denaturation steps. Therefore, SAD has an 576 essential role in determining the overall UFA content. For this reason, the relationship between SAD-related 577 genes expression and UFA composition has been studied in olive fruits, pointing out that UFA composition could be modified through the transcriptional regulation of SAD-related genes (Hernández et al., 2019). 578 579 Finally, in palm-oil fruit, the biosynthetic pathway of fatty acid has been investigated, and it was suggested 580 that the *EgWRI1-1* gene behaves as a master regulator that activates the expression of oil biosynthetic genes. 581 However, the biosynthesis of oil in palm fruits is the result of the expression of several activators and repressors transcription factors (Yeap et al., 2017). 582

583 Several biotechnological strategies aimed at incorporating additional fatty acids with healthful attributes, or 584 to modify oil composition, have been applied in different oilseed crops through genetic engineering (Haslam 585 et al., 2016; Rogalski & Carrer, 2011). Among fruit tree species, palm fruit tree has been the focus of several 586 studies for the obtainment of oil palm producing less saturated oil with an increased content of high value-587 added fatty acids, like oleic acid in the mesocarp at the expense of palmitic content (Masani et al., 2018). Biochemical studies determined two main target enzymes to be regulated for higher oleic acid content, that 588 589 are the palmitoyl-ACP thioesterase (PAT) and b-ketoacyl-ACP synthase II (KAS II), that have been 590 characterized in model plants and for which different transformation vectors have been designed and constructed with tissue specific promoters (Masani et al., 2018). The progress in regeneration and 591 592 transformation protocols for this perennial species carried out during the last two decades, and the recent 593 successful sequencing of the oil palm genome will give the opportunity to develop palm oil with modified oil 594 palm lipid metabolism. 595

596 Antinutrients

As defence strategy against predators and pathogens, plants produce some bioactive compounds, as 597 resveratrol and glucosinate, that have been demonstrated to have healthy benefits. However, among these 598 599 compounds there are also antinutritional metabolites like toxins or allergens, which are considered harmful 600 for human body (Newell-McGloughlin, 2008). Many fruit species contain allergens that cause oral allergy syndrome to consumers, associated with gastrointestinal symptoms, urticaria, and anaphylaxis. The most 601 602 sensitive individuals to birch tree pollen protein (Bet v 1) are the most subjected to allergy reactions, in fact, 603 many fruit species contain pathogenesis-related proteins homologous to Bet v 1, including Mal d 1 in apple, Pyr c 1 in pear, Pru p 1 in peach, Pru ar 1 in apricot, Pru av 1 in sweet cherry, and Fra a 1 in strawberry 604 (Aranzana et al., 2019; Guarino & Sciarrillo, 2018; Kaiser et al., 2016; Paris et al., 2017) (Table 1). Four 605 606 classes of allergens have been identified in apple (Mal d 1, Mal d 2, Mal d 3 and Mal d 4), with Mal d 1 being the most allergenic (Paris et al., 2017). The amount of Mal d 1 in apple can change according to the 607 608 cultivar. A reduction of Mal d 1 allergenicity has been observed in genotypes with high polyphenol oxidase 609 activity, which could be enhanced by increasing chlorogenic and caffeic acid in apple genotypes (Kschonsek 610 et al., 2019). A less common but more severe allergy could also be developed in people sensitive to the Mal 611 d 3 protein. Allergen levels in apple are usually not assessed during the breeding process, but some cultivars

- with low amount of allergens have been identified and reducing the content of these proteins in plants couldbe the focus of future apple breeding programs (Kaiser et al., 2020).
- 614 Strawberry is a fruit with potential allergenicity for the consumer, caused by different proteins according to 615 the geographic area. Fra a 3 and Fra a 4 proteins are the mainly responsible for allergies occurring in the
- 616 Mediterranean area, while Fra a 1 in North and Central Europe. Fruit colour has been correlated with
- 617 allergen content, therefore the *Fra a 1* and *Fra a 3* genes are linked to the flavonoid biosynthesis. The total
- 618 elimination of these gene products would lead to colourless strawberries, however, considerable variation in
- the Fra a 1 content has been described for different red coloured cultivars, suggesting that Fra a 1 and Fra a 3might not be always limiting in the flavonoid biosynthesis (Kaiser et al., 2016). Screening for strawberry
- accessions with low content of Fra a 1 and Fra a 3 could be useful to identify and breed strawberries aimed atreducing the allergen content.
- Other species present allergens, like *Actinidia* spp. and *Citrus* spp. (Table 1). In *Actinidia* spp., the protein actinidin (Act 1a) has been recognized for its allergenicity inducing tingling in the oral cavity and anaphylactic reactions. Differences in the content of this protein have been detected in different kiwifruit genotypes, and some of them showing low levels of Act 1 a were suggested as interesting breeding material for low-allergenicity fruits (Kamiyoshihara et al., 2018). The content of allergens is species-dependent, and pummelo was suggested as the species with the lower allergenicity risk, being an interesting breeding source for hypoallergenic citrus fruits (Wu et al., 2016).
- Among biotechnological tools, RNAi has been used as effective tool for downregulating genes encoding
 undesirable compounds in several plant species, like gossypol in cottonseed, or phytic acid in wheat (Newell McGloughlin, 2008; Rathore et al., 2020). RNAi strategy was successfully applied in apple by silencing the
- $Mal \ dl$ gene. In this study transgenic apple plants showed a significant reduction in $Mal \ dl$ transcript and
- 634 many allergic subjects resulted completely tolerant to these fruits (Dubois et al., 2015). In another study the 635 strawberry *Fra a 1* allergen encoding gene was downregulated through transient RNAi-mediated silencing.
- strawberry *Fra a 1* allergen encoding gene was downregulated through transient RNAi-mediated silencing,
 however the parallel reduction of the *phenylalanine ammonia lyase (FaPAL)* and *chalcone synthase (FaCHS)*
- flavonoid biosynthesis-related transcripts was observed, leading to white phenotype in the transformed fruits
 (Muñoz et al., 2010).
- 639

640 Conclusions and future perspectives

- 641 Over the years, the market demand has changed toward the obtainment of food crops with improved quality 642 and safety, through low environmental impact systems. Several experimental and epidemiological data are 643 confirming that the consumption of fruit and vegetables is related to many health benefits, decreasing the risk of chronic diseases insurgence, and reducing their huge healthcare costs. In addition, the nutritional 644 645 enhancement of food crops is of increasing interest also as sustainable and promising approach to help in 646 solving the diffused nutrients' deficiencies. Fruit tree species represent an incredible source of biologically active molecules important for the human diet, such as carbohydrates, vitamins, minerals and antioxidant 647 648 compounds. However, there is no fruit containing all the nutrient constituents and rarely these compounds 649 are uniformly distributed in all plant tissues; in addition, some agricultural practices seem to diminish gradually their level in fruit (Kaur et al., 2020a). Thus, there is the need to work on nutrients maintenance 650 651 and their target improvement.
- 652 This review summarized many of the attempts done to increase the nutritional quality of fruit tree species during the last decades through both traditional breeding associated to molecular techniques, like MAS, and 653 the most recent NBTs applied to these crops. The most accepted method for fruit biofortification is 654 conventional breeding, that offers a sustainable and cost-effective alternative to agronomic and transgenic 655 656 biofortification strategies (Garg et al., 2018). The improvement of fruit characteristics is obtained through the implementation of breeding programs for the traits of interest, and, to set up an effective breeding program, 657 658 the availability of sufficient genotypic variation is necessary. However, the genetic variation present in the 659 genetic pool of some species is sometimes limited for selected traits of interest. This issue could be solved by 660 the valorisation of wild germplasm and ancient accessions, including them in crossing programs; however, it would take long time and many crossing cycles to introduce the traits of interest into commercial cultivars. 661 The application of molecular techniques, as MAS, to conventional breeding could help in speeding up these 662 selection processes, determining more accurately and in a shorter time the genotypes carrying the genes of 663 interest. However, even if these kinds of molecular techniques significantly optimize the breeding process, 664 the timescale and efforts needed for the biofortification of some fruit tree species remain still relevant, and 665 the application of NBTs represents the most suitable alternative solution (Fig. 2). When considering the use 666
- of biotechnological tools for the modulation of a specific biosynthetic pathway and/or for the increase of a

668 phytonutrient in a target crop, several aspects need to be taken into consideration. First of all, a deep 669 knowledge on the interconnection among the different metabolic pathways is necessary, because the alteration of one pathway can have unexpected effects on one or more related ones; in this sense, the 670 671 development of effective bioinformatic and "omics" approaches, especially functional genomic studies, are 672 essential tools for elucidating such pathways (Kumar et al., 2020). In addition, other aspects must be considered, like the bioavailability of the increased nutrient, its effective dose for human diet, as well as the 673 maintenance of the other agronomical traits proper of the target cultivar, which could be negatively affected 674 675 by the alteration of metabolic fluxes. Further parameters, such as cultivation and production costs, as well as potential allergic responses of the consumer need to be considered (Uncu et al., 2013). 676

677 During the last decades a huge number of studies have been published describing the use of biotechnological approaches aimed to the enhancement of healthy compounds in plants; however, the number of released 678 679 transgenic biofortified crops still represents a very low percentage compared to those obtained by traditional 680 breeding (Garg et al., 2018). Indeed, genetically modified plants are still arising public concern and represent 681 a controversial topic for governmental agencies, especially in Europe. However, the progress made during 682 the last decade in the field of biotechnological techniques have introduced more fast, efficient and precise 683 crop improvement tools, which could arise less biosafety and regulatory concerns, and that could be considered more similar to conventional breeding techniques, like cisgenesis and intragenesis (Capriotti et al., 684 2020; Limera et al., 2017). An increasing number of studies have been published based on the use of 685 CRISPR/Cas9-mediated genome editing technology for both knock-in and knock-out of target traits also in 686 687 fruit tree species, and for few of them results showing transgene-free mutants' regeneration have been 688 reported (Kaur et al., 2020a). Among NBTs, RNAi still represents one of the most exploited biotechnological 689 approaches, especially for metabolically engineered plants, because it can lead to the modulation of a 690 transcript expression without its complete knockout. This represents an important feature when only a 691 reduced expression level of a target gene is necessary. Furthermore, RNAi represents a good compromise to 692 both reduce the risk of off-target effects, and to avoid the introduction of new allergens; indeed, no new 693 protein is introduced through this technique, but the modulation of specific genes expression is mediated by dsRNA molecules expressed in the target plant (Arpaia et al., 2020; Mezzetti et al., 2020). 694

In EU, the European Court of Justice has recently confirmed that all new NBTs are regulated by the 2001/18 directive on genetically modified organisms (GMOs), because all referring to the use of recombinant DNA technology (Schulman et al., 2020) (Fig. 2). This is negatively impacting on the approval of any new NBTs product in Europe because of the lack of acceptance by the consumers. While in other regions of the world (USA, Canada, Japan, Australia) a regulatory system based on products and not on methods has been adopted, then the approval of any new NBTs product is more facilitated (Eckerstorfer et al., 2019).

701 A novelty is offered by the exogenous application of dsRNAs on plants, namely spray-induced gene 702 silencing (SIGS), which has been mainly tested as environmental-friendly alternative to traditional pesticides for pests and pathogens control (Taning et al., 2020). Recently, these dsRNA-based products have been 703 704 classified not as GMOs but as biological pesticides (OECD, 2020). Beside the impact that this technology 705 will have on replacing traditional pesticides, RNA-based compounds for external applications are of high 706 interest to downregulate endogenous genes in plant, opening the possibility to exploit SIGS as a tool also for 707 modulating metabolic pathways in a target crop, without introducing any stable modification in the plant 708 genome (Dubrovina & Kiselev, 2019).

709 Breeding and biotechnological approaches will strongly contribute to the development of new plants-based 710 biofortified foods, but their diffusion on the market will depend on their acceptance, especially for new 711 products derived from biotechnologies, and their commercial exploitation would also be enhanced through 712 health claims, unfortunately not yet very widespread for fruits enriched with nutritional compounds

713 (Mezzetti et al., 2016). 714

715 Acknowledgments

The authors acknowledge EU financial support through iPLANTA COST Action CA 15223, MIURPRIN2017 national program via grant N.20173LBZM2-Micromolecule, "Programma di Sviluppo Rurale
della Regione Marche" 2014-2020. Misura 16.1.A.2-Project i.d. 27961 and PRIMA– Partnership for
Research and Innovation in the Mediterranean Area 2019-2022 MEDBERRY project.

721 Conflicts of interest

720

722 The authors declare no conflicts of interest in relation to this manuscript and state that the opinions expressed

723 are their own and should not be considered to reflect those of any other individuals or organizations.

724

725 References

- Abdelghafar, A., da Silva Linge, C., Okie, W. R., & Gasic, K. (2020). Mapping QTLs for phytochemical
 compounds and fruit quality in peach. *Molecular Breeding*, 40(3). https://doi.org/10.1007/s11032-020 01114-y
- Allan, A. C., & Espley, R. V. (2018). MYBs Drive Novel Consumer Traits in Fruits and Vegetables. *Trends in Plant Science*, 23(8), 693–705. https://doi.org/10.1016/j.tplants.2018.06.001
- Amah, D., Alamu, E., Adesokan, M., van Biljon, A., Maziya-dixon, B., Swennen, R., & Labuschagne, M.
 (2019b). Variability of carotenoids in a Musa germplasm collection and implications for provitamin A biofortification. *Food Chemistry*, 2, 100024. https://doi.org/10.1016/j.fochx.2019.100024
- Amah, D., van Biljon, A., Maziya-Dixon, B., Labuschagne, M., & Swennen, R. (2019a). Effects of In Vitro
 Polyploidization on Agronomic Characteristics and Fruit Carotenoid Content; Implications for Banana
 Genetic Improvement. *Frontiers in Plant Science*, 10, 1–12. https://doi.org/10.3389/fpls.2019.01450
- Aranzana, M. J., Decroocq, V., Dirlewanger, E., Eduardo, I., Gao, Z. S., Gasic, K., Iezzoni, A., Jung, S.,
 Peace, C., Prieto, H., Tao, R., Verde, I., Abbott, A. G., & Arús, P. (2019). Prunus genetics and
 applications after de novo genome sequencing: achievements and prospects. *Horticulture Research*,
 6(1). https://doi.org/10.1038/s41438-019-0140-8
- Arcos, Y., Godoy, F., Flores-Ortiz, C., Arenas-M, A., & Stange, C. (2020). Boosting carotenoid content in
 Malus domestica var. Fuji by expressing AtDXR through an Agrobacterium-mediated transformation
 method. *Biotechnology and Bioengineering*, *117*(7), 2209–2222. https://doi.org/10.1002/bit.27358
- Arpaia, S., Christiaens, O., Giddings, K., Jones, H., Mezzetti, B., Moronta-Barrios, F., Perry, J. N., Sweet, J.
 B., Taning, C. N. T., Smagghe, G., & Dietz-Pfeilstetter, A. (2020). Biosafety of GM Crop Plants
 Expressing dsRNA: Data Requirements and EU Regulatory Considerations. *Frontiers in Plant Science*,
 11, 940. https://doi.org/10.3389/fpls.2020.00940
- Arya, M., Reshama, U., Syama, S. T., Anaswara, S., & Karishama, S. (2019). *Nutraceuticals in vegetables : New breeding approaches for nutrition , food and health : A review.* 8(1), 677–682.
- Assefa, A. D., Saini, R. K., & Keum, Y. S. (2017). Fatty acids, tocopherols, phenolic and antioxidant
 properties of six citrus fruit species: a comparative study. *Journal of Food Measurement and Characterization*, *11*(4), 1665–1675. https://doi.org/10.1007/s11694-017-9546-x
- Azuma, A., Kobayashi, S., Mitani, N., Shiraishi, M., Yamada, M., Ueno, T., Kono, A., Yakushiji, H., &
 Koshita, Y. (2008). Genomic and genetic analysis of Myb-related genes that regulate anthocyanin
 biosynthesis in grape berry skin. *Theoretical and Applied Genetics*, *117*(6), 1009–1019.
 https://doi.org/10.1007/s00122-008-0840-1
- Barbey, C., Hogshead, M., Schwartz, A. E., Mourad, N., Verma, S., Lee, S., Whitaker, V. M., & Folta, K. M.
 (2020). The Genetics of Differential Gene Expression Related to Fruit Traits in Strawberry (Fragaria ×ananassa). *Frontiers in Genetics*, 10, 1–15. https://doi.org/10.3389/fgene.2019.01317
- Basson, C. E., Groenewald, J. H., Kossmann, J., Cronjé, C., & Bauer, R. (2011). Upregulation of
 pyrophosphate: Fructose 6-phosphate 1-phosphotransferase (PFP) activity in strawberry. *Transgenic Research*, 20(4), 925–931. https://doi.org/10.1007/s11248-010-9451-0
- Bobis, O., Zagrai, I., Bonta, V., Zagrai, L., Marghitas, L. A., Dezmirean, D. S., Pasca, C., & Urcan, A.
 (2017). Comparative Studies on Chemical Composition of Two Conventional Bred and One
 Genetically Engineered Plum-Fruits. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies*, 74(2), 149–156.
- Brendolise, C., Espley, R. V, Lin-Wang, K., Laing, W., Peng, Y., McGhie, T., Dejnoprat, S., Tomes, S.,
 Hellens, R. P., & Allan, A. C. (2017). Multiple Copies of a Simple MYB-Binding Site Confers Transregulation by Specific Flavonoid-Related R2R3 MYBs in Diverse Species. In *Frontiers in Plant Science* (Vol. 8, p. 1864). https://www.frontiersin.org/article/10.3389/fpls.2017.01864
- Bulley, S., Wright, M., Rommens, C., Yan, H., Rassam, M., Lin-Wang, K., Andre, C., Brewster, D.,
 Karunairetnam, S., Allan, A. C., & Laing, W. A. (2012). Enhancing ascorbate in fruits and tubers
 through over-expression of the l-galactose pathway gene GDP-l-galactose phosphorylase. *Plant Biotechnology Journal*, *10*(4), 390–397. https://doi.org/10.1111/j.1467-7652.2011.00668.x
- Cao, K., Ding, T., Mao, D., Zhu, G., Fang, W., Chen, C., Wang, X., & Wang, L. (2018). Transcriptome
 analysis reveals novel genes involved in anthocyanin biosynthesis in the flesh of peach. *Plant Physiology and Biochemistry*, *123*, 94–102. https://doi.org/10.1016/j.plaphy.2017.12.005
- 778 Capriotti, L., Baraldi, E., Mezzetti, B., Limera, C., & Sabbadini, S. (2020). Biotechnological approaches:
- Gene overexpression, gene silencing, and genome editing to control fungal and oomycete diseases in

- 780 grapevine. International Journal of Molecular Sciences, 21(16), 1–29.
- 781 https://doi.org/10.3390/ijms21165701
- Carvalho, R. F., & Folta, K. M. (2017). Assessment of promoters and a selectable marker for development of
 strawberry intragenic vectors. *Plant Cell, Tissue and Organ Culture, 128*(2), 259–271.
 https://doi.org/10.1007/s11240-016-1105-3
- 785 Çelik, F., Balta, M. F., Ercişli, S., Gündoğdu, M., Karakaya, O., & Yaviç, A. (2019). Tocopherol Contents of
 786 Almond Genetic Resources from Eastern and Western Turkey. *Erwerbs-Obstbau*, *61*(3), 257–262.
 787 https://doi.org/10.1007/s10341-019-00425-5
- 788 Cirilli, M., Bassi, D., & Ciacciulli, A. (2016). Sugars in peach fruit: a breeding perspective. *Horticulture* 789 *Research*, 3(1), 15067. https://doi.org/10.1038/hortres.2015.67
- 790 De la Rosa, R., Arias-Calderón, R., Velasco, L., & León, L. (2016). Early selection for oil quality
 791 components in olive breeding progenies. *European Journal of Lipid Science and Technology*, *118*(8),
 792 1160–1167. https://doi.org/10.1002/ejlt.201500425
- 793 De Steur, H., Blancquaert, D., Strobbe, S., Lambert, W., Gellynck, X., & Van Der Straeten, D. (2015). Status
 794 and market potential of transgenic biofortified crops. *Nature Biotechnology*, *33*(1), 25–29.
 795 https://doi.org/10.1038/nbt.3110
- Dubois, A. E. J., Pagliarani, G., Brouwer, R. M., Kollen, B. J., Dragsted, L. O., Eriksen, F. D., Callesen, O.,
 Gilissen, L. J. W. J., Krens, F. A., Visser, R. G. F., Smulders, M. J. M., Vlieg-Boerstra, B. J., FlokstraDe Blok, B. J., & Van De Weg, W. E. (2015). First successful reduction of clinical allergenicity of food
 by genetic modification: Mal d 1-silenced apples cause fewer allergy symptoms than the wild-type
 cultivar. *Allergy: European Journal of Allergy and Clinical Immunology*, *70*(11), 1406–1412.
 https://doi.org/10.1111/all.12684
- Bubrovina, A. S., & Kiselev, K. V. (2019). Exogenous RNAs for Gene Regulation and Plant Resistance.
 International Journal of Molecular Sciences, 20(9), 2282. https://doi.org/10.3390/ijms20092282
- Eckerstorfer, M. F., Engelhard, M., Heissenberger, A., Simon, S., & Teichmann, H. (2019). Plants developed
 by new genetic modification techniques-Comparison of existing regulatory frameworks in the EU and
 Non-EU countries. *Frontiers in Bioengineering and Biotechnology*, 7.
 https://doi.org/10.3389/fbioe.2019.00026
- Edge-Garza, D. A., Luby, J. J., & Peace, C. (2015). Decision support for cost-efficient and logistically
 feasible marker-assisted seedling selection in fruit breeding. *Molecular Breeding*, 35(12), 1–15.
 https://doi.org/10.1007/s11032-015-0409-z
- 811 Espley, R. V., Butts, C. A., Laing, W. A., Martell, S., Smith, H., McGhie, T. K., Zhang, J., Paturi, G.,
 812 Hedderley, D., Bovy, A., Schouten, H. J., Putterill, J., Allan, A. C., & Hellens, R. P. (2014). Dietary
 813 flavonoids from modified apple reduce inflammation markers and modulate gut microbiota in mice.
 814 *Journal of Nutrition*, 144(2), 146–154. https://doi.org/10.3945/jn.113.182659
- Espley, R. V., Hellens, R. P., Putterill, J., Stevenson, D. E., Kutty-Amma, S., & Allan, A. C. (2007). Red
 colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. *Plant Journal*, 49(3), 414–427. https://doi.org/10.1111/j.1365-313X.2006.02964.x
- Fang, T., Zhen, Q., Liao, L., Owiti, A., Zhao, L., Korban, S. S., & Han, Y. (2017). Variation of ascorbic acid
 concentration in fruits of cultivated and wild apples. *Food Chemistry*, 225, 132–137.
 https://doi.org/10.1016/j.foodchem.2017.01.014
- Fenech, M., Amaya, I., Valpuesta, V., & Botella, M. A. (2019). Vitamin C content in fruits: Biosynthesis and regulation. *Frontiers in Plant Science*, *9*, 1–21. https://doi.org/10.3389/fpls.2018.02006
- Fischer, T. C., Mirbeth, B., Rentsch, J., Sutter, C., Ring, L., Flachowsky, H., Habegger, R., Hoffmann, T.,
 Hanke, M. V., & Schwab, W. (2014). Premature and ectopic anthocyanin formation by silencing of
 anthocyanidin reductase in strawberry (Fragaria × ananassa). *New Phytologist*, 201(2), 440–451.
 https://doi.org/10.1111/nph.12528
- Fitzpatrick, T. B., & Chapman, L. M. (2020). The importance of thiamine (vitamin B1) in plant health: From crop yield to biofortification. *The Journal of Biological Chemistry*, 295(34), 12002–12013.
 https://doi.org/10.1074/jbc.REV120.010918
- Fritsche, S., Wang, X., & Jung, C. (2017). Recent advances in our understanding of tocopherol biosynthesis
 in plants: An overview of key genes, functions, and breeding of vitamin E improved crops.
 Antioxidants, 6(4). https://doi.org/10.3390/antiox6040099
- B33 Gao, Q., Luo, H., Li, Y., Liu, Z., & Kang, C. (2020). Genetic modulation of RAP alters fruit coloration in
 both wild and cultivated strawberry. *Plant Biotechnology Journal*, *18*(7), 1550–1561.
 https://doi.org/10.1111/pbi.13317

- B36 García-Gómez, B. E., Salazar, J. A., Dondini, L., Martínez-Gómez, P., & Ruiz, D. (2019). Identification of
 QTLs linked to fruit quality traits in apricot (Prunus armeniaca L.) and biological validation through
 gene expression analysis using qPCR. *Molecular Breeding*, 39(2), 28. https://doi.org/10.1007/s11032018-0926-7
- Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., & Arora, P. (2018). Biofortified
 Crops Generated by Breeding, Agronomy, and Transgenic Approaches Are Improving Lives of
 Millions of People around the World. *Frontiers in Nutrition*, *5*, 12.
- 843 https://doi.org/10.3389/fnut.2018.00012
- Georgiadou, E. C., Goulas, V., Ntourou, T., Manganaris, G. A., Kalaitzis, P., & Fotopoulos, V. (2016).
 Regulation of on-tree vitamin E biosynthesis in olive fruit during successive growing years: The impact of fruit development and environmental cues. *Frontiers in Plant Science*, 7, 1–12.
 https://doi.org/10.3389/fpls.2016.01656
- Giampieri, F., Gasparrini, M., Forbes-Hernandez, T. Y., Mazzoni, L., Capocasa, F., Sabbadini, S., AlvarezSuarez, J. M., Afrin, S., Rosati, C., Pandolfini, T., Molesini, B., Sánchez-Sevilla, J. F., Amaya, I.,
 Mezzetti, B., & Battino, M. (2018). Overexpression of the Anthocyanidin Synthase Gene in Strawberry
 Enhances Antioxidant Capacity and Cytotoxic Effects on Human Hepatic Cancer Cells. *Journal of Agricultural and Food Chemistry*, *66*(3), 581–592. https://doi.org/10.1021/acs.jafc.7b04177
- Guarino, C., & Sciarrillo, R. (2018). The identification of allergen proteins in two different varieties of
 strawberry by two different approaches: Proteomic and western blotting method. *Annals of Agricultural Sciences*, 63(2), 181–189. https://doi.org/10.1016/j.aoas.2018.11.003
- Haslam, R. P., Sayanova, O., Kim, H. J., Cahoon, E. B., & Napier, J. A. (2016). Synthetic redesign of plant
 lipid metabolism. *The Plant Journal : For Cell and Molecular Biology*, 87(1), 76–86.
 https://doi.org/10.1111/tpj.13172
- Hernández, M. L., Belaj, A., Sicardo, M. D., León, L., de la Rosa, R., Martín, A., Martínez-Rivas, J. M., &
 Atienza, S. G. (2017). Mapping quantitative trait loci controlling fatty acid composition in olive. *Euphytica*, 213(1). https://doi.org/10.1007/s10681-016-1802-3
- Hernández, M. L., Sicardo, M. D., Alfonso, M., & Martínez-Rivas, J. M. (2019). Transcriptional regulation
 of stearoyl-acyl carrier protein desaturase genes in response to abiotic stresses leads to changes in the
 unsaturated fatty acids composition of olive mesocarp. *Frontiers in Plant Science*, 10, 1–12.
 https://doi.org/10.3389/fpls.2019.00251
- Hossain, M. R., Kim, H. T., Shanmugam, A., Nath, U. K., Goswami, G., Song, J. Y., Park, J. I., & Nou, I. S.
 (2018). Expression profiling of regulatory and biosynthetic genes in contrastingly anthocyanin rich
 strawberry (Fragaria × ananassa) cultivars reveals key genetic determinants of fruit color. *International Journal of Molecular Sciences*, 19(3), 1–20. https://doi.org/10.3390/ijms19030656
- Iamjud, K., Srimat, S., Sangwanangkul, P., Wasee, S., & Thaipong, K. (2016). Antioxidant properties and
 fruit quality of selected papaya breeding lines. *ScienceAsia*, 42(5), 332–339.
 https://doi.org/10.2306/scienceasia1513-1874.2016.42.332
- Jiang, C. C., Zhang, Y. F., Lin, Y. J., Chen, Y., & Lu, X. K. (2019). Illumina® sequencing reveals candidate
 genes of carotenoid metabolism in three pummelo cultivars (Citrus maxima) with different pulp color. *International Journal of Molecular Sciences*, 20(9). https://doi.org/10.3390/ijms20092246
- Kadomura-Ishikawa, Y., Miyawaki, K., Takahashi, A., & Noji, S. (2015). RNAi-mediated silencing and
 overexpression of the FaMYB1 gene and its effect on anthocyanin accumulation in strawberry fruit.
 Biologia Plantarum, 59(4), 677–685. https://doi.org/10.1007/s10535-015-0548-4
- Kafkas, E., Burgut, A., Ozcan, H., Ozcan, A., Sutyemez, M., Kafkas, S., & Türemis, N. (2017). Fatty Acid,
 Total Phenol and Tocopherol Profiles of Some Walnut Cultivars: A Comparative Study. *Food and Nutrition Sciences*, 08(12), 1074–1084. https://doi.org/10.4236/fns.2017.812079
- Kaiser, N., Douches, D., Dhingra, A., Glenn, K. C., Herzig, P. R., Stowe, E. C., & Swarup, S. (2020). The
 role of conventional plant breeding in ensuring safe levels of naturally occurring toxins in food crops.
 Trends in Food Science and Technology, *100*, 51–66. https://doi.org/10.1016/j.tifs.2020.03.042
- Kaiser, R., Mageney, V., Schwefel, K., Vollmers, D., Krüger, A., & Horn, R. (2016). Genotyping of red and
 white fruited strawberry (Fragaria L.) accessions and hybrids based on microsatellite markers and on
 the genetic diversity in the allergen genes fra a 1 and fra a 3. *Genetic Resources and Crop Evolution*,
 63(7), 1203–1217. https://doi.org/10.1007/s10722-015-0311-x
- Kamiyoshihara, Y., Nakamura, T., Itagaki, Y., Asada, S., Aoki, T., Mizuno, S., Watanabe, K., Inoue, H., &
 Tateishi, A. (2018). Differential constitution in promoter region leads to a phenotype with a lower
 allergic actinidin level in yellow-fleshed kiwifruit (Actinidia chinensis). *Horticulture Journal*, 87(2),

- 892 288–296. https://doi.org/10.2503/hortj.OKD-133
- Kanchiswamy, C. N., Sargent, D. J., Velasco, R., Maffei, M. E., & Malnoy, M. (2015). Looking forward to
 genetically edited fruit crops. In *Trends in Biotechnology* (Vol. 33, Issue 2, pp. 62–64). Elsevier Ltd.
 https://doi.org/10.1016/j.tibtech.2014.07.003
- Karanjalker, G., & Begane, N. (2016). Breeding Perennial Fruit Crops for Quality Improvement. *Erwerbs- Obstbau*, 58(2), 119–126. https://doi.org/10.1007/s10341-015-0264-4
- Katoch, R., & Thakur, N. (2013). Advances in RNA interference technology and its impact on nutritional
 improvement, disease and insect control in plants. *Applied Biochemistry and Biotechnology*, 169(5),
 1579–1605. https://doi.org/10.1007/s12010-012-0046-5
- 801 Kaur, N., Alok, A., Shivani, Kumar, P., Kaur, N., Awasthi, P., Chaturvedi, S., Pandey, P., Pandey, A.,
 902 Pandey, A. K., & Tiwari, S. (2020b). CRISPR/Cas9 directed editing of lycopene epsilon-cyclase
 903 modulates metabolic flux for β-carotene biosynthesis in banana fruit. *Metabolic Engineering*,
 904 59(January), 76–86. https://doi.org/10.1016/j.ymben.2020.01.008
- Kaur, N., Awasthi, P., & Tiwari, S. (2020a). Fruit crops improvement using CRISPR/Cas9 system. In
 Genome Engineering via CRISPR-Cas9 System. Elsevier Inc. https://doi.org/10.1016/b978-0-12 818140-9.00012-x
- 908 Kim, M., Kim, S. C., Song, K. J., Kim, H. B., Kim, I. J., Song, E. Y., & Chun, S. J. (2010). Transformation
 909 of carotenoid biosynthetic genes using a micro-cross section method in kiwifruit (Actinidia deliciosa cv.
 910 Hayward). *Plant Cell Reports*, 29(12), 1339–1349. https://doi.org/10.1007/s00299-010-0920-y
- 911 Kiselev, K. V., & Dubrovina, A. S. (2020). Overexpression of stilbene synthase genes to modulate the
 912 properties of plants and plant cell cultures. *Biotechnology and Applied Biochemistry*.
 913 https://doi.org/10.1002/bab.1884
- 814 Kobayashi, S., Ding, C. K., Nakamura, Y., Nakajima, I., & Matsumoto, R. (2000). Kiwifruits (Actinidia deliciosa) transformed with a Vitis stilbene synthase gene produce piceid (resveratrol-glucoside). *Plant Cell Reports*, 19(9), 904–910. https://doi.org/10.1007/s002990000203
- 917 Krens, F. A., Schaart, J. G., van der Burgh, A. M., TinnenbroekCapel, I. E. M., Groenwold, R., Kodde, L. P.,
 918 Broggini, G. A. L., Gessler, C., & Schouten, H. J. (2015). Cisgenic apple trees; development,
 919 characterization, and performance. *Frontiers in Plant Science*, 6(APR).
 920 https://doi.org/10.3389/fpls.2015.00286
- 921 Kschonsek, J., Dietz, A., Wiegand, C., Hipler, U. C., & Böhm, V. (2019). Allergenicity of apple allergen
 922 Mal d 1 as effected by polyphenols and polyphenol oxidase due to enzymatic browning. *Lwt*, *113*,
 923 108289. https://doi.org/10.1016/j.lwt.2019.108289
- Kui, L. W., Liu, Y., Espley, R. V., Karunairetnam, S., McGhie, T. K., Hellens, R. P., & Allan, A. C. (2014).
 Regulation of anthocyanin biosynthesis in strawberry (Fragaria sp.) by over-expression of a key
 transcription factor. *Acta Horticulturae*, 1048, 137–142.
- Kumar, G. B. S., Srinivas, L., & Ganapathi, T. R. (2011). Iron fortification of banana by the expression of
 soybean ferritin. *Biological Trace Element Research*, 142(2), 232–241. https://doi.org/10.1007/s12011010-8754-6
- 930 Kumar, P., Shaunak, I., & Verma, M. L. (2020). *Chapter 6 Biotechnological application of health*931 *promising bioactive molecules* (M. L. Verma & A. K. B. T.-B. P. of B. C. Chandel (eds.); pp. 165–189).
 932 Elsevier. https://doi.org/10.1016/B978-0-444-64323-0.00006-0
- Lachowicz, S., Oszmiański, J., & Pluta, S. (2017). The composition of bioactive compounds and antioxidant
 activity of Saskatoon berry (Amelanchier alnifolia Nutt.) genotypes grown in central Poland. *Food Chemistry*, 235, 234–243. https://doi.org/10.1016/j.foodchem.2017.05.050
- Leng, X., Wang, P., Wang, C., Zhu, X., Li, X., Li, H., Mu, Q., Li, A., Liu, Z., & Fang, J. (2017). Genomewide identification and characterization of genes involved in carotenoid metabolic in three stages of
 grapevine fruit development. *Scientific Reports*, 7(1), 1–13. https://doi.org/10.1038/s41598-017-040040
- Li, M., Li, P., Ma, F., Dandekar, A. M., & Cheng, L. (2018a). Sugar metabolism and accumulation in the
 fruit of transgenic apple trees with decreased sorbitol synthesis. *Horticulture Research*, 5(1).
 https://doi.org/10.1038/s41438-018-0064-8
- Li, X. G., Yang, T.T., Yu, Z.Y., Huo, J.W., Dong, Q., Duan, Y.D., & Yang, G. (2019a). Cloning of flavonoid
 3',5'-hydroxylase and 3'-hydroxylase homologs from black currant (Ribes nigrum) and their differential
 expression at various fruit maturation stages. *Journal of Forestry Research*, 30(2), 463–470.
 https://doi.org/10.1007/s11676-017-0560-y
- 947 Li, X., Jin, L., Pan, X., Yang, L., & Guo, W. (2019b). Proteins expression and metabolite profile insight into

- 948 phenolic biosynthesis during highbush blueberry fruit maturation. *Food Chemistry*, 290, 216–228.
 949 https://doi.org/10.1016/j.foodchem.2019.03.115
- Li, Y., Fang, J., Qi, X., Lin, M., Zhong, Y., Sun, L., & Cui, W. (2018b). Combined analysis of the fruit
 metabolome and transcriptome reveals candidate genes involved in flavonoid biosynthesis in Actinidia
 arguta. *International Journal of Molecular Sciences*, 19(5), 1–17. https://doi.org/10.3390/ijms19051471
- Limera, C., Sabbadini, S., Sweet, J. B., & Mezzetti, B. (2017). New Biotechnological Tools for the Genetic
 Improvement of Major Woody Fruit Species. In *Frontiers in Plant Science* (Vol. 8, p. 1418).
 https://doi.org/https://doi.org/10.3389/fpls.2017.01418
- Lin-Wang, K., McGhie, T. K., Wang, M., Liu, Y., Warren, B., Storey, R., Espley, R. V., & Allan, A. C.
 (2014). Engineering the anthocyanin regulatory complex of strawberry (Fragaria vesca). *Frontiers in Plant Science*, 5, 1–14. https://doi.org/10.3389/fpls.2014.00651
- Liu, W., Wang, Y., Yu, L., Jiang, H., Guo, Z., Xu, H., Jiang, S., Fang, H., Zhang, J., Su, M., Zhang, Z., Chen, X., Chen, X., & Wang, N. (2019). MdWRKY11 Participates in Anthocyanin Accumulation in RedFleshed Apples by Affecting MYB Transcription Factors and the Photoresponse Factor MdHY5. *Journal of Agricultural and Food Chemistry*, 67(32), 8783–8793.
 https://doi.org/10.1021/acs.jafc.9b02920
- Ma, B., Zhao, S., Wu, B., Wang, D., Peng, Q., Owiti, A., Fang, T., Liao, L., Ogutu, C., Korban, S. S., Li, S.,
 & Han, Y. (2015). Construction of a high density linkage map and its application in the identification of QTLs for soluble sugar and organic acid components in apple. *Tree Genetics & Genomes*, *12*(1), 1.
 https://doi.org/10.1007/s11295-015-0959-6
- Mann, J., Cummings, J. H., Englyst, H. N., Key, T., Liu, S., Riccardi, G., Summerbell, C., Uauy, R., van
 Dam, R. M., Venn, B., Vorster, H. H., & Wiseman, M. (2007). FAO/WHO Scientific Update on
 carbohydrates in human nutrition: Conclusions. *European Journal of Clinical Nutrition*, *61*, S132–S137.
 https://doi.org/10.1038/sj.ejcn.1602943
- Masani, M. Y. A., Izawati, A. M. D., Rasid, O. A., & Parveez, G. K. A. (2018). Biotechnology of oil palm:
 Current status of oil palm genetic transformation. *Biocatalysis and Agricultural Biotechnology*,
 15(January), 335–347. https://doi.org/10.1016/j.bcab.2018.07.008
- 975 Mazzoni, L., Di Vittori, L., Balducci, F., Forbes-Hernández, T. Y., Giampieri, F., Battino, M., Mezzetti, B.,
 976 & Capocasa, F. (2020). Sensorial and nutritional quality of inter and intra—Specific strawberry
 977 genotypes selected in resilient conditions. *Scientia Horticulturae*, 261, 108945.
 978 https://doi.org/10.1016/j.scienta.2019.108945
- Mazzoni, L., Giampieri, F., Alvarez Suarez, J. M., Gasparrini, M., Mezzetti, B., Forbes Hernandez, T. Y.,
 Battino, M. A. (2019) Isolation of strawberry anthocyanin-rich fractions and their mechanisms of action
 against murine breast cancer cell lines. *Food & Function*, 10(11), 7103-7120. doi: 10.1039/c9fo01721f.
- McCallum, J., Laing, W., Bulley, S., Thomson, S., Catanach, A., Shaw, M., Knaebel, M., Tahir, J., Deroles,
 S., Timmerman-Vaughan, G., Crowhurst, R., Hilario, E., Chisnall, M., Lee, R., Macknight, R., & Seal,
 A. (2019). Molecular characterisation of a supergene conditioning super-high vitamin c in kiwifruit
 hybrids. *Plants*, 8(7), 1–24. https://doi.org/10.3390/plants8070237
- 986 Mccallum, S., Simpson, C., & Graham, J. (2018). Raspberry. *Raspberry*, 121–144.
 987 https://doi.org/10.1007/978-3-319-99031-6
- Mezzetti, B., Balducci, F., Capocasa, F., Zhong, C. F., Cappelletti, R., Di Vittori, L., Mazzoni, L., Giampieri,
 F., & Battino, M. (2016). Breeding Strawberry for Higher Phytochemicals Content and Claim It: Is It
 Possible? *International Journal of Fruit Science*, *16*(1), 194–206.
 https://doi.org/10.1080/15538362.2016.1250695
- Mezzetti, B. (2013). Breeding and biotechnology for improving the nutritional quality of strawberry. In
 Journal of Berry Research (Vol. 3, Issue 3, pp. 127–133). https://doi.org/10.3233/JBR-130053
- Mezzetti, B., Smagghe, G., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., Kostov, K.,
 Sabbadini, S., Opsahl-Sorteberg, H.-G., Ventura, V., Taning, C. N. T., & Sweet, J. (2020). RNAi: What
 is its position in agriculture? *Journal of Pest Science*, 93(4), 1125–1130.
 https://doi.org/10.1007/s10340-020-01238-2
- Migicovsky, Z., & Myles, S. (2017). Exploiting wild relatives for genomics-assisted breeding of perennial
 crops. In *Frontiers in Plant Science* (Vol. 8, p. 460). https://doi.org/10.3389/fpls.2017.00460
- 1000 Muñoz, C., Hoffmann, T., Escobar, N. M., Ludemann, F., Botella, M. A., Valpuesta, V., & Schwab, W.
- 1001 (2010). The strawberry fruit fra a allergen functions in flavonoid biosynthesis. *Molecular Plant*, 3(1), 113–124. https://doi.org/10.1093/mp/ssp087
- 1003 Naing, A. H., & Kim, C. K. (2018). Roles of R2R3-MYB transcription factors in transcriptional regulation of

- anthocyanin biosynthesis in horticultural plants. *Plant Molecular Biology*, 98(1–2), 1–18.
 https://doi.org/10.1007/s11103-018-0771-4
- 1006 Nantawan, U., Kanchana-Udomkan, C., Bar, I., & Ford, R. (2019). Linkage mapping and quantitative trait
 1007 loci analysis of sweetness and other fruit quality traits in papaya. *BMC Plant Biology*, *19*(1), 1–11.
 1008 https://doi.org/10.1186/s12870-019-2043-0
- Newell-McGloughlin, M. (2008). Nutritionally improved agricultural crops. *Plant Physiology*, *147*(3), 939–
 953. https://doi.org/10.1104/pp.108.121947
- 1011 OECD. (2020). Considerations for the Environmental Risk Assessment of the Application of Sprayed or
 1012 Externally Applied ds-RNA-Based Pesticides.
- 1013 http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2020)26&docla
 1014 nguage=en
- Paris, R., Pagliarani, G., Savazzini, F., Aloisi, I., Iorio, R. A., Tartarini, S., Ricci, G., & Del Duca, S. (2017).
 Comparative analysis of allergen genes and pro-inflammatory factors in pollen and fruit of apple
 varieties. *Plant Science*, 264(July), 57–68. https://doi.org/10.1016/j.plantsci.2017.08.006
- Paul, J. Y., Khanna, H., Kleidon, J., Hoang, P., Geijskes, J., Daniells, J., Zaplin, E., Rosenberg, Y., James, A.,
 Mlalazi, B., Deo, P., Arinaitwe, G., Namanya, P., Becker, D., Tindamanyire, J., Tushemereirwe, W.,
 Harding, R., & Dale, J. (2017). Golden bananas in the field: elevated fruit pro-vitamin A from the
 expression of a single banana transgene. *Plant Biotechnology Journal*, *15*(4), 520–532.
 https://doi.org/10.1111/pbi.12650
- Peng, Y., Thrimawithana, A. H., Cooney, J. M., Jensen, D. J., Espley, R. V, & Allan, A. C. (2020). The
 proanthocyanin-related transcription factors MYBC1 and WRKY44 regulate branch points in the
 kiwifruit anthocyanin pathway. *Scientific Reports*, 10(1), 14161. https://doi.org/10.1038/s41598-02070977-0
- Peraza-Magallanes, A. Y., Pereyra-Camacho, M. A., Sandoval-Castro, E., Medina-Godoy, S., Valdez Morales, M., Clegg, M. T., & Calderón-Vázquez, C. L. (2017). Exploring genetic variation, oil and α tocopherol content in avocado (Persea americana) from northwestern Mexico. *Genetic Resources and Crop Evolution*, 64(3), 443–449. https://doi.org/10.1007/s10722-016-0478-9
- Pérez, A. G., León, L., Pascual, M., de la Rosa, R., Belaj, A., & Sanz, C. (2019). Analysis of olive (Olea
 europaea L.) genetic resources in relation to the content of Vitamin e in virgin olive oil. *Antioxidants*, 8(8). https://doi.org/10.3390/antiox8080242
- Pons, E., Alquézar, B., Rodríguez, A., Martorell, P., Genovés, S., Ramón, D., Rodrigo, M. J., Zacarías, L., &
 Peña, L. (2014). Metabolic engineering of β-carotene in orange fruit increases its in vivo antioxidant
 properties. *Plant Biotechnology Journal*, *12*(1), 17–27. https://doi.org/10.1111/pbi.12112
- Pott, D. M., Osorio, S., & Vallarino, J. G. (2019). From central to specialized metabolism: An overview of
 some secondary compounds derived from the primary metabolism for their role in conferring
 nutritional and organoleptic characteristics to fruit. *Frontiers in Plant Science*, 10.
 https://doi.org/10.3389/fpls.2019.00835
- Proietti, S., Moscatello, S., Villani, F., Mecucci, F., Walker, R. P., Famiani, F., & Battistelli, A. (2019).
 Quality and nutritional compounds of Prunus Cerasus L. Var. austera fruit grown in central Italy.
 HortScience, 54(6), 1005–1012. https://doi.org/10.21273/HORTSCI13960-19
- Qi, Y., Zhu, C., Chen, J., Liu, G., Yang, Z., & Chen, W. (2019). Comparative analysis of the quality and
 health-promoting compounds of two-shaped fruits of wild Lycium ruthenicum Murr. from the Qinghai–
 Tibet Plateau. *Acta Physiologiae Plantarum*, 41(6), 1–10. https://doi.org/10.1007/s11738-019-2888-8
- Ramírez-Briones, E., Rodríguez Macías, R., Casarrubias Castillo, K., del Río, R. E., Martínez-Gallardo, N.,
 Tiessen, A., Ordaz-Ortiz, J., Cervantes-Hernández, F., Délano-Frier, J. P., & Zañudo-Hernández, J.
 (2019). Fruits of wild and semi-domesticated Diospyros tree species have contrasting phenological,
 metabolic, and antioxidant activity profiles. *Journal of the Science of Food and Agriculture*, 99(13),
 6020–6031. https://doi.org/10.1002/jsfa.9878
- Rathore, K. S., Pandeya, D., Campbell, L. M., Wedegaertner, T. C., Puckhaber, L., Stipanovic, R. D.,
 Thenell, J. S., Hague, S., & Hake, K. (2020). Ultra-Low Gossypol Cottonseed: Selective Gene
 Silencing Opens Up a Vast Resource of Plant-Based Protein to Improve Human Nutrition. *Critical Reviews in Plant Sciences*, *39*(1), 1–29. https://doi.org/10.1080/07352689.2020.1724433
- 1056 Ricci, A., Sabbadini, S., Prieto, H., Padilla, I. M., Dardick, C., Li, Z., Scorza, R., Limera, C., Mezzetti, B.,
 1057 Perez-Jimenez, M., Burgos, L., & Petri, C. (2020). Genetic Transformation in Peach (Prunus persica L.):
 1058 Challenges and Ways Forward. In *Plants* (Vol. 9, Issue 8). https://doi.org/10.3390/plants9080971
- 1059 Rodriguez-Concepcion, M., Avalos, J., Bonet, M. L., Boronat, A., Gomez-Gomez, L., Hornero-Mendez, D.,

- 1060 Limon, M. C., Meléndez-Martínez, A. J., Olmedilla-Alonso, B., Palou, A., Ribot, J., Rodrigo, M. J.,
- 1061 Zacarias, L., & Zhu, C. (2018). A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. Progress in Lipid Research, 70(February), 62-93. 1062 1063 https://doi.org/10.1016/j.plipres.2018.04.004
- 1064 Rogalski, M., & Carrer, H. (2011). Engineering plastid fatty acid biosynthesis to improve food quality and
- biofuel production in higher plants. Plant Biotechnology Journal, 9(5), 554-564. 1065 https://doi.org/10.1111/j.1467-7652.2011.00621.x 1066
- 1067 Rühmann, S., Treutter, D., Fritsche, S., Briviba, K., & Szankowski, I. (2006). Piceid (resveratrol glucoside) synthesis in stilbene synthase transgenic apple fruit. Journal of Agricultural and Food Chemistry, 1068 1069 54(13), 4633-4640. https://doi.org/10.1021/jf0602491
- 1070 Ryu, J., Kwon, S.-J., Jo, Y. D., Choi, H.-I., Kang, K.-Y., Nam, B. mi, Kim, D.-G., Jin, C.-H., Kim, J.-B., 1071 Kim, E.-Y., Oh, S. C., Ha, B.-K., & Kang, S.-Y. (2017). Fruit Quality and Chemical Contents of 1072 Hybrid Boysenberry (Rubus ursinus) Lines Developed by Hybridization and Gamma Irradiation. Plant Breeding and Biotechnology, 5(3), 228-236. https://doi.org/10.9787/pbb.2017.5.3.228 1073
- Sabbadini, S., Capriotti, L., Molesini, B., Pandolfini, T., Navacchi, O., Limera, C., Ricci, A., & Mezzetti, B. 1074 1075 (2019). Comparison of regeneration capacity and Agrobacterium-mediated cell transformation efficiency of different cultivars and rootstocks of Vitis spp. via organogenesis. Scientific Reports, 9(1). 1076 1077 https://doi.org/10.1038/s41598-018-37335-7
- Saito, T. (2016). Advances in Japanese pear breeding in Japan. Breeding Science, 66(1), 46–59. 1078 1079 https://doi.org/10.1270/jsbbs.66.46
- 1080 Schneeman, B. O. (2001). Featured article linking agricultural production and human nutrition. In Journal of 1081 the Science of Food and Agriculture (Vol. 81, Issue 1, pp. 3-9). https://doi.org/10.1002/1097-0010(20010101)81:1<3::AID-JSFA743>3.0.CO;2-Q 1082
- 1083 Schouten, H. J., Krens, F. A., & Jacobsen, E. (2006). Cisgenic plants are similar to traditionally bred plants. 1084 EMBO Reports, 7(8), 750-753. https://doi.org/10.1038/sj.embor.7400769
- 1085 Schulman, A. H., Oksman-Caldentey, K. M., & Teeri, T. H. (2020). European Court of Justice delivers no justice to Europe on genome-edited crops. *Plant Biotechnology Journal*, 18(1), 8–10. 1086 1087 https://doi.org/10.1111/pbi.13200
- 1088 Sebastiani, L., & Busconi, M. (2017). Recent developments in olive (Olea europaea L.) genetics and 1089 genomics: applications in taxonomy, varietal identification, traceability and breeding. Plant Cell 1090 Reports, 36(9), 1345-1360. https://doi.org/10.1007/s00299-017-2145-9
- 1091 Seo, Y. S., Kim, S. J., Harn, C. H., & Kim, W. T. (2011). Ectopic expression of apple fruit homogentisate phytyltransferase gene (MdHPT1) increases tocopherol in transgenic tomato (Solanum lycopersicum cv. 1092 1093 Micro-Tom) leaves and fruits. Phytochemistry, 72(4-5), 321-329. 1094 https://doi.org/10.1016/j.phytochem.2010.12.013
- Shimelis, H., & Laing, M. (2012). Timelines in conventional crop improvement: Pre-breeding and breeding 1095 1096 procedures. Australian Journal of Crop Science, 6(11), 1542–1549.
- Song, L., Wang, X., Han, W., Qu, Y., Wang, Z., Zhai, R., Yang, C., Ma, F., & Xu, L. (2020). PBMYB120 1097 1098 negatively regulates anthocyanin accumulation in pear. International Journal of Molecular Sciences, 1099 21(4). https://doi.org/10.3390/ijms21041528
- Stanys, V., Bendokas, V., Rugienius, R., Sasnauskas, A., Frercks, B., Mažeikienė, I., & Šikšnianas, T. (2019). 1100 Management of anthocyanin amount and composition in genus Ribes using interspecific hybridisation. 1101 1102 Scientia Horticulturae, 247, 123–129. https://doi.org/10.1016/j.scienta.2018.12.014
- Stokstad, E. (2019). After 20 years, Golden Rice nears approval. Science, 366(6468), 934 LP 934. 1103
- 1104 https://doi.org/10.1126/science.366.6468.934
- Strobbe, S., De Lepeleire, J., & Van Der Straeten, D. (2018). From in planta function to vitamin-rich food 1105 1106 crops: The ACE of biofortification. Frontiers in Plant Science, 871, 1–27. 1107 https://doi.org/10.3389/fpls.2018.01862
- Sugiyama, A., Omura, M., Shimada, T., Fujii, H., Endo, T., Shimizu, T., Nesumi, H., Nonaka, K., & Ikoma, 1108 1109 Y. (2014). Expression quantitative trait loci analysis of carotenoid metabolism-related genes in citrus. 1110 *Journal of the Japanese Society for Horticultural Science*, 83(1), 32–43.
- 1111 https://doi.org/10.2503/jjshs1.CH-054
- Surya, M., Suhartati, S., Ismaini, L., Lusini, Y., Destri, D., Anggraeni, D., Normasiwi, S., Asni, N., & Sidiq, 1112 M. (2018). Fruit Nutrients of Five Species of Wild Raspberries (Rubus spp.) from Indonesian
- 1113
- 1114 Mountain's Forests. Journal of Tropical Life Science, 8(1), 75-80.
- 1115 https://doi.org/10.11594/jtls.08.01.13

- Taning, C. N. T., Arpaia, S., Christiaens, O., Dietz-Pfeilstetter, A., Jones, H., Mezzetti, B., Sabbadini, S.,
 Sorteberg, H.-G., Sweet, J., Ventura, V., & Smagghe, G. (2020). RNA-based biocontrol compounds:
 current status and perspectives to reach the market. *Pest Management Science*, *76*(3), 841–845.
 https://doi.org/10.1002/ps.5686
- Uncu, A. O., Doganlar, S., & Frary, A. (2013). Biotechnology for Enhanced Nutritional Quality in Plants.
 Critical Reviews in Plant Sciences, *32*(5), 321–343. https://doi.org/10.1080/07352689.2013.781453
- Urrutia, M., Schwab, W., Hoffmann, T., & Monfort, A. (2015). Genetic dissection of the (poly)phenol
 profile of diploid strawberry (Fragaria vesca) fruits using a NIL collection. *Plant Science*, 242, 151–168.
 https://doi.org/10.1016/j.plantsci.2015.07.019
- Wang, J. H., Liu, J. J., Chen, K. L., Li, H. W., He, J., Guan, B., & He, L. (2016b). Anthocyanin Biosynthesis
 Regulation in the Fruit of Citrus sinensis cv. Tarocco. *Plant Molecular Biology Reporter*, 34(6), 1043–
 1055. https://doi.org/10.1007/s11105-016-0984-0
- Wang, L. F., Qi, X. X., Huang, X. S., Xu, L. L., Jin, C., Wu, J., & Zhang, S. L. (2016a). Overexpression of
 sucrose transporter gene PbSUT2 from Pyrus bretschneideri, enhances sucrose content in Solanum
 lycopersicum fruit. *Plant Physiology and Biochemistry*, *105*, 150–161.
 https://doi.org/10.1016/j.plaphy.2016.04.019
- Wang, L., Tang, W., Hu, Y., Zhang, Y., Sun, J., Guo, X., Lu, H., Yang, Y., Fang, C., Niu, X., Yue, J., Fei, Z.,
 & Liu, Y. (2019). A MYB/bHLH complex regulates tissue-specific anthocyanin biosynthesis in the
 inner pericarp of red-centered kiwifruit Actinidia chinensis cv. Hongyang. *Plant Journal*, 99(2), 359–378. https://doi.org/10.1111/tpj.14330
- Wang, N., Jiang, S., Zhang, Z., Fang, H., Xu, H., Wang, Y., & Chen, X. (2018). Malus sieversii: the origin,
 flavonoid synthesis mechanism, and breeding of red-skinned and red-fleshed apples. *Horticulture Research*, 5(1). https://doi.org/10.1038/s41438-018-0084-4
- Wang, Z., Cui, Y., Vainstein, A., Chen, S., & Ma, H. (2017). Regulation of fig (Ficus carica L.) fruit color:
 Metabolomic and transcriptomic analyses of the flavonoid biosynthetic pathway. *Frontiers in Plant Science*, 8. https://doi.org/10.3389/fpls.2017.01990
- Watkins, J. L., & Pogson, B. J. (2020). Prospects for Carotenoid Biofortification Targeting Retention and Catabolism. *Trends in Plant Science*, 25(5), 501–512. https://doi.org/10.1016/j.tplants.2019.12.021
- Wu, J., Chen, L., Lin, D., Ma, Z., & Deng, X. (2016). Development and Application of a Multiplex RealTime PCR Assay as an Indicator of Potential Allergenicity in Citrus Fruits. *Journal of Agricultural and Food Chemistry*, 64(47), 9089–9098. https://doi.org/10.1021/acs.jafc.6b03410
- Xie, X. B., Li, S., Zhang, R. F., Zhao, J., Chen, Y. C., Zhao, Q., Yao, Y. X., You, C. X., Zhang, X. S., & Hao,
 Y. J. (2012). The bHLH transcription factor MdbHLH3 promotes anthocyanin accumulation and fruit
 colouration in response to low temperature in apples. *Plant, Cell and Environment*, *35*(11), 1884–1897.
 https://doi.org/10.1111/j.1365-3040.2012.02523.x
- Yeap, W. C., Lee, F. C., Shabari Shan, D. K., Musa, H., Appleton, D. R., & Kulaveerasingam, H. (2017).
 WRI1-1, ABI5, NF-YA3 and NF-YC2 increase oil biosynthesis in coordination with hormonal signaling during fruit development in oil palm. *Plant Journal*, *91*(1), 97–113.
 https://doi.org/10.1111/tpj.13549
- Yousef, G. G., Brown, A. F., Guzman, I., Ballington, J. R., & Lila, M. A. (2016). Variations in chlorogenic
 acid levels in an expanded gene pool of blueberries. *AIMS Agriculture and Food*, 1(3), 357–368.
 https://doi.org/10.3934/agrfood.2016.3.357
- Yu, J. Q., Gu, K. Di, Sun, C. H., Zhang, Q. Y., Wang, J. H., Ma, F. F., You, C. X., Hu, D. G., & Hao, Y. J.
 (2020). The apple bHLH transcription factor MdbHLH3 functions in determining the fruit
 carbohydrates and malate. *Plant Biotechnology Journal*, 1–15. https://doi.org/10.1111/pbi.13461
- Zamany, A. J., Samadi, G. R., Kim, D. H., Keum, Y. S., & Saini, R. K. (2017). Comparative Study of Tocopherol Contents and Fatty Acids Composition in Twenty Almond Cultivars of Afghanistan. *JAOCS, Journal of the American Oil Chemists' Society*, *94*(6), 805–817.
 https://doi.org/10.1007/s11746-017-2989-8
- Zhai, R., Zhao, Y., Wu, M., Yang, J., Li, X., Liu, H., Wu, T., Liang, F., Yang, C., Wang, Z., Ma, F., & Xu, L.
 (2019). The MYB transcription factor PbMYB12b positively regulates flavonol biosynthesis in pear
 fruit. *BMC Plant Biology*, *19*(1), 1–11. https://doi.org/10.1186/s12870-019-1687-0
- Zhen, Q., Fang, T., Peng, Q., Liao, L., Zhao, L., Owiti, A., & Han, Y. (2018). Developing gene-tagged
 molecular markers for evaluation of genetic association of apple SWEET genes with fruit sugar
 accumulation. *Horticulture Research*, 5(1), 14. https://doi.org/10.1038/s41438-018-0024-3
- 1171 Zheng, X., Guliano, G., & Al-Babili, S. (2020). Carotenoid biofortification in crop plants: citius, altius,

- fortius. In *Biochimica et Biophysica Acta Molecular and Cell Biology of Lipids*.
 https://doi.org/10.1016/j.bbalip.2020.158664
- 1174 Zheng, X., Tang, Y., Ye, J., Pan, Z., Tan, M., Xie, Z., Chai, L., Xu, Q., Fraser, P. D., & Deng, X. (2019).
 1175 SLAF-Based Construction of a High-Density Genetic Map and Its Application in QTL Mapping of 1176 Carotenoids Content in Citrus Fruit. *Journal of Agricultural and Food Chemistry*, 67(3), 994–1002.
 1177 https://doi.org/10.1021/acs.jafc.8b05176
- 1178 Zhong, C. F., Mazzoni, L., Balducci, F., Di Vittori, L., Capocasa, F., Giampieri, F., & Mezzetti, B. (2017).
 1179 Evaluation of vitamin C content in fruit and leaves of different strawberry genotypes. *Acta*1180 *Horticulturae*, *1156*, 371–378. https://doi.org/10.17660/ActaHortic.2017.1156.56
- 1181 Zhou, D., Shen, Y., Zhou, P., Fatima, M., Lin, J., Yue, J., Zhang, X., Chen, L. Y., & Ming, R. (2019).
 1182 Papaya CpbHLH1/2 regulate carotenoid biosynthesis-related genes during papaya fruit ripening.
 1183 *Horticulture Research*, 6(1). https://doi.org/10.1038/s41438-019-0162-2
- Zhu, C., Sanahuja, G., Yuan, D., Farré, G., Arjó, G., Berman, J., Zorrilla-López, U., Banakar, R., Bai, C.,
 Pérez-Massot, E., Bassie, L., Capell, T., & Christou, P. (2013). Biofortification of plants with altered
 antioxidant content and composition: Genetic engineering strategies. *Plant Biotechnology Journal*, *11*(2), 129–141. https://doi.org/10.1111/j.1467-7652.2012.00740.x

1190 Figure Captions

1191

1193

1188 1189

1192 Fig. 1. Plant components of dietary interest and their main health effects.



1194 1195

Fig. 2. Diagram showing different conventional (left part) and biotechnological (right part) strategies applied
 for fruit trees biofortification. For each technique, main advantages, outcomes, public acceptance and
 expected results are reported.

1199

CONVENTIONAL / NEW BREEDING TECHNIQUES AND PRODUCTS					
Breeding	Mutagenesis	Trans/cis/intra genesis	Gene editing	Gene silencing (RNAi)	
Genetic resources (wild and cultivated germplasms)	Chemical and physical mutagenic techniques	Functional genomic studies and identification of target regulatory genes for nutritional/antinutritional compounds			
Long term in vivo selection / molecular markers (MAS, QTLs)		Short term selection of genetically modified biofortified cultivar			
Patenting biofo	ortified cultivars	GMO biosafety rules before patenting biofortified cultivars			
Public acceptance wit	thout biosafety issues	Biosafety issues and lack of public acceptance			
NEW BIOFORTIFIED CULTIVAR					

HEALTH CLAIM ON PLANT-DERIVED BIOFORTIFIED FRUITS/PRODUCTS

Table 1. Candidate genes for the improvement of nutritional traits in fruit trees characterized by conventional molecular tools or genetic engineering strategies

Compound	Fruit tree species	Candidate genes	References
		Phenolic compounds	
Flavonoids	Grapevine	VvMYBA1, VvMYBA2, VvMYB4 (-)	(Azuma et al., 2008)
	Pear	PcMYB10	(Brendolise et al., 2017)
		<i>PbMYB120</i> (-)	(Song et al., 2020)
	Apple	MdMYBA, MdMYB1, MdMYB10	(Espley et al., 2007; Krens et al., 2015; Naing & Kim, 2018)
		MdWRKY11	(Liu et al., 2019)
		MdbHLH3	(Xie et al., 2012)
		MdMYB16 (-), MdMYB17 (-), MdMYB111 (-)	(Liu et al., 2019)
	Bayberry	MrMYB1	(Naing & Kim, 2018)
	Chinese berry	MrMYB1	(Naing & Kim, 2018)
	Litchi	LcMYB1	(Naing & Kim, 2018)
	Kiwifruit	AcMYB110a	(Naing & Kim, 2018)
		MYBC1, WRKY44	(Peng et al., 2020)
		AaF3H, AaLDOX, AaUFGT AaMYB, AabHLH	(Li et al., 2018b)
	Blood orange	CsRuby	(Naing & Kim, 2018)
	Mangosteen	GmMYB10	(Naing & Kim, 2018)

	Peach	Prupe.5G105700, Prupe.5G105800,	(Abdelghafar et al.,
		Prupe.5G116/00 Pr.PL PrMVP101	2020)
		PPBL, PPMIBI0.1	(Cao et al., 2018)
	D1 1	DFR, F3H, F3 5 H, ANS	$(Q_1 \text{ et al.}, 2019)$
	Blood orange	Cs6g1/5/0, Cs5g31400, Cs9g04810	(Wang et al., 2016b)
	Sweet cherry	PavMYB10	(Aranzana et al., 2019)
	Fig	R2R3-MYB	(Wang et al., 2017)
	Blackcurrant	F3H, F3 '5 'H	(Li et al., 2019a)
	Blueberry	F3H, F3'5'H, VcUFGALT, VcUFGT73,	(Li et al., 2019b)
	·	VcU5GT, VcAPRR2, VcbHLH3, VcWD, VcWD	
	Raspberry	bHLH, NAM/CUC2 like protein, bZIP	(Mccallum et al., 2018)
	Strawberry	FvMYB10, FaMYB10, FaMYB5	(Kui et al., 2014; Lin- Wang et al., 2014)
		ANS	(Giampieri et al., 2018)
		RAP	(Gao et al., 2020)
		ANR (-)	(Fischer et al., 2014)
		FabHLH3, FabHLH3-delta, FaMYB11 (-), FaMYB9 (-), FabHLH33 (-), FaWD44-1 (-)	(Hossain et al., 2018)
		FaMYB1 (-)	(Kadomura-Ishikawa et
			al., 2015)
		Fra a 1, Fra a 3	(Kaiser et al., 2016;
	_		Muñoz et al., 2010)
Flavonols	Pear	<i>PbMYB12b</i> , <i>PbCHSb</i> , <i>PbFLS</i>	(Zhai et al., 2019)
Stilbenes	Grapevine	STS	(Kiselev & Dubrovina,
		17 1	$\frac{2020}{(W_{\rm ell}, \dots, 1)} = 4 \times 1$
		VSTI	(Kobayashi et al.,
			2000, Kummann et al., 2006)
		Vitamins	2000)
Vitamin E	Avocado	VTE3, VTE4	(Peraza-Magallanes et
			al., 2017)
	Olive	VTE1, VTE2, VTE3, VTE4, VTE5, HPPD,	(Georgiadou et al.,
		HGGT, GGR	2016)
Vitamin A	Banana	PSY2	(Paul et al., 2017)
Vitamin C	Apple	MdGGP1, MdDHAR3-3, MdNAT7-2	(Fang et al., 2017)
		MdHPT1	(Seo et al., 2011)
	K1w1fru1t	qAsA26.1 (QTL)	(McCallum et al.,
		CCD	2019)
	G: 1		(Bulley et al., 2012)
	Strawberry	FanGalUK, FanMDAK, FanGPX, FanGK, FanSODM	(Barbey et al., 2020)
Minerals		TunsoDM	
Zinc. Iron	Banana	Ferritin from Sovbean	(Kumar et al., 2011)
Terpenes		<i>,</i>	
Carotenoids	Strawberry	FanPSY, FanZDS	(Barbey et al., 2020)
	Citrus spp.	PSY, ZDS, PDS, HYb, ZEP	(Sugiyama et al., 2014)
	11		
		LYCB2/E, CCD4, NCED2, AAo3, CvP707A1	(Jiang et al., 2019)
	Papaya	CpCYC-B, CpLCY-B, CpPDS2, CpZDS,	(Zhou et al., 2019)
	~ ~	CpLCY-E, CpCHY-B	
	Apricot	LOX 2, CCD1, CCD4	(García-Gómez et al.,

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		_		2019)
Banana $PSY2$ $LCY-\varepsilon (-)$ (Paul et al., 2017) (Kaur et al., 2020b) (Kaur et al., 2020b) (Kaur et al., 2020b) (Kaur et al., 2020b) (Kaur et al., 2020b) (Arcos et al., 2020) (Pons et al., 2010) (Arcos et al., 2014)Total soluble sugarsPear SUS Apple(Saito, 2016) MdSWEET15a, MdSWEET9bFructoseStrawberry $MdbHLH3$ PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseApple $MdbHLH3$ PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseApple $MdbHLH3$ PFP from Giardia lamblia(Sebastiani & Busconi, 2017) (Basson et al., 2011)SucroseApple $MoPR$ (-)(Li et al., 2018a)Dicic acidOlive $OeSAD2$ SAD Oil palm(Sebastiani & Busconi, 2017) (Hernández et al., 2017) FAD7AllergensApple $Mal d 1, Mal d 2, Mal d 3, Mal d 4$ $Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 2.03(Dubois et al., 2015; Paris et al., 2017)(Aranzana et al., 2019)(Aranzana et al., 2019)(Aranzana et al., 2019)(Kimifruit(Dubois et al., 2017)(Karniyoshihara et al., 2018)$		Grapevine	VvDXS, VvDXR, VvGGPPS, VvPSY, VvCCD	(Leng et al., 2017)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		Banana	PSY2	(Paul et al., 2017)
Kiwifruit Apple Orange $GGPS, PDS, ZDS, CHXADXRCS\beta-CHX (-)(Kim et al., 2010)(Arcos et al., 2020)(Pons et al., 2014)Total solublesugarsPearAppleSUSMdSWEET15a, MdSWEET9b(Zhen et al., 2018)Total solublesugarsPearAppleMdSWEET15a, MdSWEET9bPFP from Giardia lamblia(Yu et al., 2020)(Basson et al., 2011)FructoseStrawberryMdbHLH3PFP from Giardia lamblia(Yu et al., 2020)(Basson et al., 2011)SucroseAppleA6PR (-)PEar(Li et al., 2018a)Dicic acidOliveOeSAD2SADOil palm(Sebastiani & Busconi,2017)(Hernández et al.,2017)Linoleic acidOliveOeFAD2-2FAD7(Sebastiani et al., 2018)Linoleic acidOliveOeFAD2-2Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01,Pru p 2.02, Pru p 2.03(Dubois et al., 2017)(Harnández et al.,2017)AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4Pru p 3, Pru p 4.02, Pru p 2.03(Dubois et al., 2017)(Aranzan et al., 2019)KiwifruitAct 1 a(Guarino & Sciarrillo,2018)(Clarino et al.,2018)$			LCY-є (-)	(Kaur et al., 2020b)
Apple OrangeAtDXR $Cs\beta$ -CHX (-)(Arcos et al., 2020) (Pons et al., 2014)CarbohydratesTotal soluble sugarsPear AppleSUS MdSWEET15a, MdSWEET9b(Zhen et al., 2018)FructoseStrawberryMdbHLH3 PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseAppleMdbHLH3 PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseAppleA6PR (-)(Li et al., 2018a)PearPbSUT2(Wang et al., 2016a)LipidsOliveOeSAD2 (Sebastiani & Busconi, 2017) (Hernández et al., 2019)Oleic acidOliveOeFAD2-2 (Masani et al., 2018)Linoleic acidOliveOeFAD2-2 (Remández et al., 2017) (Hernández et al., 2017)AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4 (Pru p 3, Pru p 4.02, Pru p 2.01, Pru p 2.01, Pru p 2.03, Pru p 2.03, Pru p 2.01, Pru p 2.03, Pru p 2.03KiwifruitAct 1 a(Guarino & Sciarrillo, 2018)		Kiwifruit	GGPS, PDS, ZDS, CHX	(Kim et al., 2010)
Orange $Cs\beta-CHX (-)$ (Pons et al., 2014)CarbohydratesTotal soluble sugarsPear SUS (Saito, 2016)Apple $MdSWEET15a, MdSWEET9b$ (Zhen et al., 2018)FructoseStrawberry PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseApple $A6PR (-)$ (Li et al., 2018a)Pear $PbSUT2$ (Wang et al., 2016a)LipidsOlive $OeSAD2$ (Sebastiani & Busconi, 2017)Oleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017)AllergensApple $Mal d 1, Mal d 2, Mal d 3, Mal d 4$ Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03AllergensApple $Mal d 1, Act 1 a$ (Guarino & Sciarrillo, 2018)		Apple	AtDXR	(Arcos et al., 2020)
CarbohydratesTotal soluble sugarsPear AppleSUS MdSWEET15a, MdSWEET9b(Saito, 2016)FructoseAppleMdSWEET15a, MdSWEET9b(Zhen et al., 2018)FructoseStrawberry PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseApple $A6PR$ (-)(Li et al., 2018a)Pear $PbSUT2$ (Wang et al., 2016a)LipidsOlive $OeSAD2$ (Sebastiani & Busconi, 2017)Oleic acidOlive $OeSAD2$ (Sebastiani & Busconi, 2017)Oil palm $PAT, KAS II$ (Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017)AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4 Pru p 3, Pru p 4.02, Pru p 2.01, Pru p 2.03StrawberryFra a 1 (Guarino & Sciarrillo, 2018)(Guarino & Sciarrillo, 2018)KiwifruitAct 1 a (Kamiyoshihara et al., 2018)		Orange	$Cs\beta$ -CHX (-)	(Pons et al., 2014)
Total soluble sugarsPear SUS (Saito, 2016)sugarsApple $MdSWEET15a, MdSWEET9b$ (Zhen et al., 2018)FructoseStrawberry PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseApple $A6PR$ (-)(Li et al., 2018a)Pear $PbSUT2$ (Wang et al., 2016a)LipidsOlive $OeSAD2$ (Sebastiani & Busconi, 2017) SAD(Sebastiani & Busconi, 2017) (Hernández et al., 2019)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017) FAD7(Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017) (Harnández et al., 2017)(Masani et al., 2017) (Harnández et al., 2017)AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4 Pru p 2, 04, Pru p 4, 01, Pru p 7, Pru p 2, 03(Dubois et al., 2017) (Aranzana et al., 2019)KiwifruitAct 1 a(Guarino & Sciarrillo, 2018)(Stamiyoshihara et al., 2018)			Carbohydrates	
AppleMdSWEET15a, MdSWEET9b(Zhen et al., 2018)FructoseStrawberry $MdbHLH3$ PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseApple $A6PR$ (-)(Li et al., 2018a)PearPbSUT2(Wang et al., 2016a)Lipids $Olive$ $OeSAD2$ SAD Oil palm(Sebastiani & Busconi, 2017) SAD Oil palmLinoleic acidOlive $OeFAD2-2$ FAD7(Sebastiani & Busconi, 2017) (Hernández et al., 2018)Linoleic acidOlive $OeFAD2-2$ FAD7(Sebastiani & Busconi, 2017) (Harnández et al., 2017) (Harnández et al., 2017)AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4 Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03(Guarino & Sciarrillo, 2018)KiwifruitAct 1 a(Kamiyoshihara et al., 2018)	Total soluble sugars	Pear	SUS	(Saito, 2016)
FructoseStrawberry $MdbHLH3$ PFP from Giardia lamblia(Yu et al., 2020) (Basson et al., 2011)SucroseApple $A6PR$ (-)(Li et al., 2018a)Pear $PbSUT2$ (Wang et al., 2016a)LipidsOlive $OeSAD2$ SAD Olive(Sebastiani & Busconi, 2017) (Hernández et al., 2019)Oleic acidOlive $OeSAD2$ SAD Oli palm(Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ FAD7(Sebastiani & Busconi, 2017) (Hernández et al., 2019)Linoleic acidOlive $OeFAD2-2$ FAD7(Sebastiani & Busconi, 2017) (Hernández et al., 2017)AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4 Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03(Guarino & Sciarrillo, 2018; Muñoz et al., 2019)KiwifruitAct 1 a(Kamiyoshihara et al., 2018)		Apple	MdSWEET15a, MdSWEET9b	(Zhen et al., 2018)
FructoseStrawberry PFP from Giardia lamblia(Basson et al., 2011)SucroseApple $A6PR$ (-)(Li et al., 2018a)Pear $PbSUT2$ (Wang et al., 2016a)Lipids $Olive$ $OeSAD2$ (Sebastiani & Busconi, 2017)Oleic acidOlive $OeSAD2$ (Sebastiani & Busconi, 2017)Oil palm $PAT, KAS II$ (Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017)FAD7(Hernández et al., 2017)FAD7(Hernández et al., 2017)Peach $Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.03(Guarino & Sciarrillo, 2017)KiwifruitAct 1 a(Guarino & Sciarrillo, 2018)$			MdbHLH3	(Yu et al., 2020)
SucroseApple $A6PR$ (-)(Li et al., 2018a)Pear $PbSUT2$ (Wang et al., 2016a)Lipids $Varget al., 2016a$ (Sebastiani & Busconi, 2017)Oleic acidOlive $OeSAD2$ (Sebastiani & Busconi, 2017) SAD (Hernández et al., 2019)(Masani et al., 2018)Dil palm $PAT, KAS II$ (Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017) $FAD7$ (Hernández et al., 2017)(Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017) $FAD7$ $Paris et al., 2017)$ (Hernández et al., 2017) $Paris et al., 2017)$ $Paris et al., 2017)$ (Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03)Strawberry $Fra a 1$ (Guarino & Sciarrillo, 2018; Muñoz et al., 2010)Kiwifruit $Act 1 a$ (Kamiyoshihara et al., 2018)	Fructose	Strawberry	PFP from Giardia lamblia	(Basson et al., 2011)
PearPbSUT2(Wang et al., 2016a)Lipids $Olive$ $OeSAD2$ (Hernández et al., 2017) SAD Oil palm(Sebastiani & Busconi, 2017) (Hernández et al., 2019) Oil palmLinoleic acidOlive $OeFAD2-2$ (FAD7(Sebastiani & Busconi, 2017) (Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017) (Hernández et al., 2017) (Hernández et al., 2017) (Hernández et al., 2017)AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4 Pru p 2,04, Pru p 4,01, Pru p 7, Pru p 2,01, Pru p 2,01, Pru p 3, Pru p 4,02, Pru p 2,03(Guarino & Sciarrillo, 2018; Muñoz et al., 2019) (Aranzana et al., 2010) (KiwifruitKiwifruitAct 1 a(Kamiyoshihara et al., 2018)	Sucrose	Apple	A6PR (-)	(Li et al., 2018a)
LipidsOleic acidOlive $OeSAD2$ (Sebastiani & Busconi, 2017)SAD(Hernández et al., 2019)Oil palm $PAT, KAS II$ (Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017)FAD7(Hernández et al., 2017)FAD7(Hernández et al., 2017)PartensisAntinutrientsAllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4(Dubois et al., 2015; Paris et al., 2017)PeachPru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 2.03(Guarino & Sciarrillo, 2018; Muñoz et al., 2019)StrawberryFra a 1(Guarino & Sciarrillo, 2018; Muñoz et al., 2010)KiwifruitAct 1 a(Kamiyoshihara et al., 2018)		Pear	PbSUT2	(Wang et al., 2016a)
Oleic acidOlive $OeSAD2$ (Sebastiani & Busconi, 2017) (Hernández et al., 2019) (Masani et al., 2019)Oil palm $PAT, KAS II$ (Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ $FAD7$ (Sebastiani & Busconi, 2017) (Hernández et al., 2017) (Hernández et al., 2017)AllergensApple $Mal d 1, Mal d 2, Mal d 3, Mal d 4$ $Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 2.03(Dubois et al., 2015; Paris et al., 2017)(Aranzana et al., 2017)(Aranzana et al., 2019)Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03StrawberryFra a 1(Guarino & Sciarrillo, 2018; Muñoz et al., 2010)(KiwifruitKiwifruitAct 1 a(Kamiyoshihara et al., 2018)$	Lipids	011		
SAD(Hernández et al., 2019)Oil palm $PAT, KAS II$ (Masani et al., 2018)Linoleic acidOlive $OeFAD2-2$ (Sebastiani & Busconi, 2017) $FAD7$ (Hernández et al., 2017)AntinutrientsAllergensApple $Mal d 1, Mal d 2, Mal d 3, Mal d 4$ (Dubois et al., 2017)Peach $Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03(Guarino & Sciarrillo, 2018)StrawberryFra a 1(Guarino & Sciarrillo, 2018; Muñoz et al., 2010)KiwifruitAct 1 a(Kamiyoshihara et al., 2018)$	Oleic acid	Olive	OeSAD2	(Sebastiani & Busconi, 2017)
Oil palmPAT, KAS II(Masani et al., 2018)Linoleic acidOliveOeFAD2-2(Sebastiani & Busconi, 2017)FAD7(Hernández et al., 2017)AntinutrientsAntinutrientsAllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4(Dubois et al., 2015; Paris et al., 2017)PeachPru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03(Guarino & Sciarrillo, 2018; Muñoz et al., 2019)StrawberryFra a 1(Guarino & Sciarrillo, 2018; Muñoz et al., 2010)(Kamiyoshihara et al., 2018)			SAD	(Hernández et al., 2019)
Linoleic acidOliveOeFAD2-2(Sebastiani & Busconi, 2017)FAD7(Hernández et al., 2017)AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4(Dubois et al., 2015; Paris et al., 2017)PeachPru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03(Guarino & Sciarrillo, 2018; Muñoz et al., 2019)StrawberryFra a 1(Guarino & Sciarrillo, 2018; Muñoz et al., 2010)(Kamiyoshihara et al., 2018)		Oil palm	PAT, KAS II	(Masani et al., 2018)
FAD7(Hernández et al., 2017)AntinutrientsAllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4(Dubois et al., 2015; Paris et al., 2017)PeachPru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03(Aranzana et al., 2019)StrawberryFra a 1(Guarino & Sciarrillo, 2018; Muñoz et al., 2010)KiwifruitAct 1 a(Kamiyoshihara et al., 2018)	Linoleic acid	Olive	OeFAD2-2	(Sebastiani & Busconi, 2017)
AntinutrientsAllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4(Dubois et al., 2015; Paris et al., 2017)PeachPru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03(Aranzana et al., 2019)StrawberryFra a 1(Guarino & Sciarrillo, 2018; Muñoz et al., 			FAD7	(Hernández et al., 2017)
AllergensAppleMal d 1, Mal d 2, Mal d 3, Mal d 4(Dubois et al., 2015; Paris et al., 2017)PeachPru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03(Aranzana et al., 2019)StrawberryFra a 1(Guarino & Sciarrillo, 2018; Muñoz et al., 			Antinutrients	
Peach Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03 (Aranzana et al., 2019) Strawberry Fra a 1 (Guarino & Sciarrillo, 2018; Muñoz et al., 2010) Kiwifruit Act 1 a (Kamiyoshihara et al., 2018)	Allergens	Apple	Mal d 1, Mal d 2, Mal d 3, Mal d 4	(Dubois et al., 2015; Paris et al., 2017)
StrawberryFra a l(Guarino & Sciarrillo, 2018; Muñoz et al., 2010)KiwifruitAct l a(Kamiyoshihara et al., 2018)		Peach	Pru p 2.04, Pru p 4.01, Pru p 7, Pru p 2.01, Pru p 3, Pru p 4.02, Pru p 2.02, Pru p 2.03	(Aranzana et al., 2019)
KiwifruitAct l a(Kamiyoshihara et al., 2018)		Strawberry	Fra a 1	(Guarino & Sciarrillo, 2018; Muñoz et al., 2010)
)		Kiwifruit	Act 1 a	(Kamiyoshihara et al., 2018)
<i>Citrus</i> spp. <i>Cit s 1.01, Cit s 2.01, Cit s 3.01</i> (Wu et al., 2016)		Citrus spp.	Cit s 1.01, Cit s 2.01, Cit s 3.01	(Wu et al., 2016)

(-) = negative regulator