

## Exploring and exploiting anthocyanins for human needs: potato as a research case study

Annalisa Staiti<sup>1</sup>, Vincenzo D'Amelia<sup>2\*</sup>, Domenico Carputo<sup>1</sup>

<sup>1</sup> Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Italy

<sup>2</sup> National Research Council (CNR), Institute of Biosciences and Bioresources (IBBR), Via Università 133, 80055 Portici, Italy

\* Corresponding author: [vincenzo.damelia@ibbr.cnr.it](mailto:vincenzo.damelia@ibbr.cnr.it); Tel.; +39-081-2539587

Received: 20 June 2022; Accepted: 7 September; Published: 21 September 2022

**Abstract:** Anthocyanins are a group of polyphenolic water-soluble pigments, largely distributed in the plant kingdom. Being natural pigments with a health-promoting bioactivity, there is an increasing interest for their application in food and drugs industry. The increasing in anthocyanins production is an enticing achievement of industries. In the first part of this review, we highlight key concepts related to the biochemistry, biological function and genetics of these important pigments in the potato. We chose this crop because it displays a valuable anthocyanin chemical diversity and it is also highly amenable to various biotechnological applications. In this latter regard, we present and briefly discuss the potential of cell cultures as a suitable method for the production of highly decorated anthocyanins. The final message of this review is to underline the impact of bio-factories as a customizable and sustainable strategy for anthocyanin production and their high potential in industrial and medical applications.

**Keywords:** Metabolic engineering, plant cell cultures, MYB transcription factors, flavonoids biosynthesis, *Solanum tuberosum*.

### 1. Anthocyanins: an overture on the biotechnological methods for their production

Plant secondary metabolites are a large group of chemical compounds which are distributed in almost all plant organs. Despite their definition as “secondary”, these molecules play a fundamental role in plant development, propagation and survival. For example, the coloration of flowers and fruits helps to attract pollinators and seed dispersers and is crucial for plant reproduction under natural conditions (Davies et al., 2012; Landi et al., 2015). For their important role in plant physiology today the term “specialized” is preferred over secondary metabolites (Pichersky et al., 2011). Among specialized metabolites, anthocyanins (from Greek ανθος (anthos) ‘= flower and κυανός (kyanos) ‘= blue), belonging to flavonoids family, are intensely studied for their antimicrobial, antioxidant, and free radical scavenging properties that have applications in food technology, human health and plant protection (D’Amelia et al., 2018). They accumulate in a wide array of plant families including *Rosaceae* (e.g. *Fragaria vesca* and *Rubus fruticosus*), *Moraceae* (e.g. *Ficus carica* and *Morus nigra*), *Brassicaceae* (e.g. *Brassica oleracea* and *Raphanus sativus*), *Poaceae* (e.g. *Oryza sativa* and *Zea mays*), *Solanaceae* (e.g. *Solanum melongena*, *Capsicum annuum*, and *Solanum tuberosum*). As anthocyanins are found in a large variety of plant tissues, it stands to reason that one of the main approaches used for their production is through extraction and isolation from fresh matrices or from food industry by-products (Silva et al., 2017). However, many factors as seasonality, unstableness due to environmental conditions, and large space requirements for cropping often negatively impact the production of these metabolites. In this scenario, biotechnologies may help to overcome limits and constraints, providing reliable ways to efficiently and effectively produce and extract anthocyanins. For example, microbial production by

engineered microorganisms is an alternative strategy that has been described in detail by Marienhagen and Bott (2013), Cress et al. (2017) ‘and Levisson et al. (2018). It combines fast growth and easy cultivation with a trouble-free manipulation technique (Zha et al., 2020). However, microorganisms used for this purpose, mainly yeasts (as *Saccharomyces cerevisiae*) ‘and *Escherichia coli*, require the expression of at least 11 transgenes to produce the simplest anthocyanin molecule, the pelargonidin 3-O-glucoside, starting from phenylalanine (Dudnik et al., 2018). The number of transgenes required increases with the level of decorations for the target anthocyanins; for example, more than 20 transgenes are required for acetylated anthocyanins with a blue tint (Appelhagen et al., 2018). An oldie but goodie strategy that overcomes these limits is the use of plant cell platforms. Indeed, unlike microbial cells, plant cells contain all the genetic information required to produce and store anthocyanins at the vacuole level. In the last few years, several European biotech companies as Arterra Biosciences ([www.arterrabio.it](http://www.arterrabio.it)) ‘and Mibelle Biochemistry ([www.mibellebiochemistry.com](http://www.mibellebiochemistry.com)) ‘developed robust plant cell cultures, obtaining a consistent amount of anthocyanins used as additives in cosmetic products. Further plant cell culture technology-derived active cosmetic ingredients, currently available on the market are reviewed by Georgiev et al., 2017.

Plant cells culture technologies have been explored also to produce anthocyanin with pharmaceutical and clinical interest. Appelhagen et al. (2018) ‘suggested that cell cultures in stir tank bioreactors can be a suitable system for the production of <sup>13</sup>C-labelled anthocyanins, which are required for human stable-isotope tracer and bioavailability studies. Recent findings on anthocyanins and the potential and limits of biotechnological strategies or new applications in crop breeding and management are reviewed by Kallscheuer et al., 2019; Bhaskar et al., 2020; Marone et al., 2022. The main objective of this paper is not to review recent findings on anthocyanin characterization and applications, but rather to focus on the exploitation of the different hues enhancing their production using molecular farming. Based on this, with the potato as a model crop and cell culture as a valuable strategy, we first report the current state of art on anthocyanin biosynthesis and their relevance in food technology and human nutrition. Then, we discuss potential biotechnological strategies focusing on anthocyanin production through potato cell culture platforms.

## **2. Anthocyanin exploitation: potato as a research case study**

As the third crop of worldwide importance for human consumption, the cultivated potato *S. tuberosum* has long been a central concern for research on anthocyanin diversity due to the presence of a large germplasm showing tubers with contrasting colors (De Jong et al., 2014; Liu et al., 2015; Oertel et al., 2017). Among *Solanaceae* crops, the potato is probably the richest source of anthocyanins in terms of quality and quantity. Potato also possesses the largest anthocyanin chemical diversity and, what is more, its anthocyanins are highly glycosyl acylated (Zhao et al., 2017). This last characteristic enhances the resistance of its anthocyanins to physiochemical and biochemical stressors (e.g. temperature and light, ROS, pH variations, digestive enzymes) ‘(Khoo et al., 2017). Moreover, the potato offers unique opportunities for genetics studies and biotechnological applications to investigate anthocyanin regulatory mechanisms and to design strategies for anthocyanin production. Some of the advantages in using potato for basic research and more applicative studies on anthocyanins are reported hereafter. The genome sequence of the cultivated potato (Potato Genome Sequencing Consortium, 2011), as well as that of some tuber-bearing wild potato species (Aversano et al., 2015; Leisner et al., 2018), is available. Several tetraploid potato RNA-Seq datasets have been deposited to public repositories and are a useful instrument for de novo transcriptome assembly (Ramšak et al., 2021). Potato stands out among all other crops also for the value of its germplasm, characterized by high genetic diversity, availability, and utility; six genebanks hold 41% of the global potato accessions, i.e. The French National Institute for Agricultural Research (INRA) ‘in France (11%), Vavilov Institute in Russia (9%), The International Potato Center (CIP) ‘in Peru (8%), The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) ‘in Germany (5%), USDA-ARS in the USA (5%), and The National Institute of Agrobiological Sciences

(NIAS) ‘in Japan (3%)’ (Peralta et al., 2021). Methods for stable and transient expression of noteworthy genes are also well developed (Ma et al., 2017; Dangol et al., 2019; Hofvander et al., 2021). Further, CRISPR/Cas technologies have opened new avenues in genome editing of the potato, reducing potential off-targets and obtaining stably inherited transgene-free plants after genetic segregation. The potato is also highly amenable to cell culture, reacting in a “textbook manner”. Indeed, several studies reported the employment of embryo culture, somatic hybridization *via* protoplast cultures, and somatic cell selection to circumvent interspecific incompatibility and to select resistance to several biotic and abiotic stresses (Barrell et al., 2013; Tiwari et al., 2019). Potato cell cultures provide a valuable platform to deepen the molecular mechanisms behind the regulation of specialized metabolites and to produce biologically important molecules (Davies and Deroles, 2014). Through the orchestrated applications of these innovative techniques, potato has not only the potential to provide an additional proportion of the food intake for the increasing global population, but it can also be an interesting resource for pharmaceutical and food industries that are interested in using plant-derived molecules with important bioactive properties.

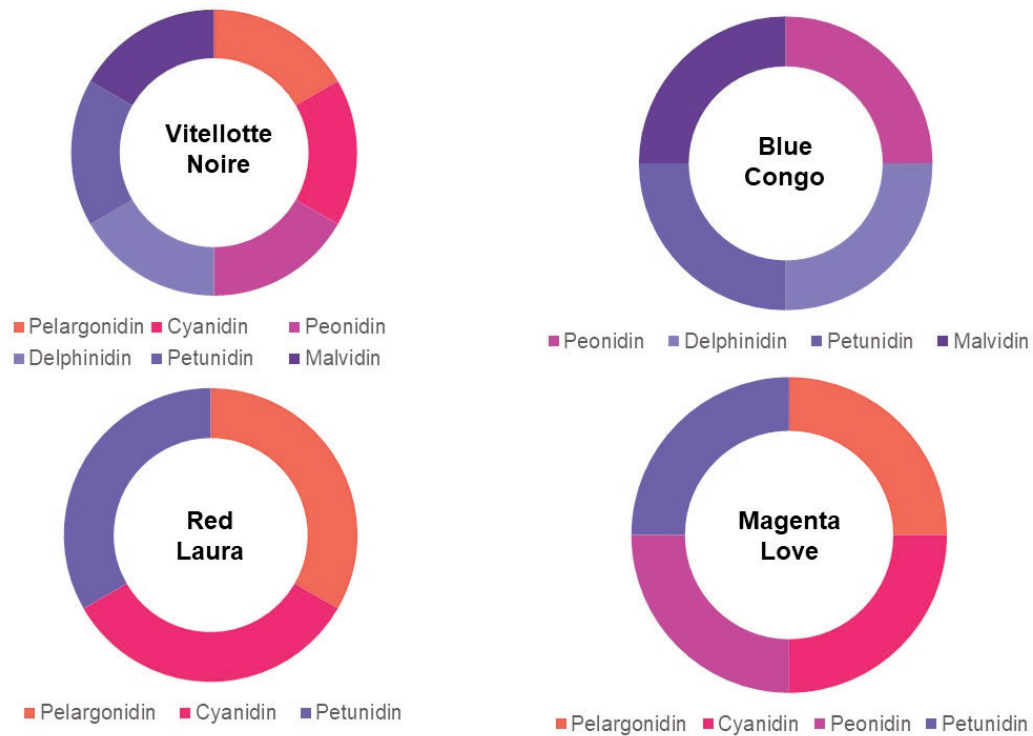
### 3. Anthocyanin biochemistry and genetic regulation in *Solanum tuberosum*

#### 3.1. Structural variation and classification of anthocyanins

More than 600 anthocyanins have been identified in nature (Andersen et al., 2005). They derive from six main anthocyanidins, namely pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Liu et al., 2018). Among them, the primary anthocyanidins are: pelargonidin, which produces the colors orange, pink and red; delphinidin, which produces the colors purple, blue, or dark blue; and cyanidin, which produces red or mauve colors and it is found mainly in fruits. Anthocyanins consist of a molecule of benzene fused with a molecule of pyran, connected in turn to a phenolic group, which can have different substituent groups: hydroxylic (-OH) ‘or methyl (-CH<sub>3</sub>). This complex molecule takes the name of “flavylium cation” and it is the basic structure of all anthocyanins. In addition to the different structures of the anthocyanidins, the molecule can be conjugated to different sugar moieties which transform anthocyanidins in anthocyanins. Finally, the hydroxylic group (-OH) ‘of the glycosyl substituents can be acylated with organic acids (as p-coumaric, caffeic, and ferulic acids) ‘via ester bonds, thus obtaining acylated anthocyanins. This aspect is important as some decorations (as acylation, glycosylation and methylation) ‘to the anthocyanin aglycone provide specific functions that can overcome important functions and drawbacks that anthocyanins can have. Indeed, several studies have confirmed that glycosyl acylations increase the ability of anthocyanins to tolerate various physicochemical (light, heat, pH changes) ‘and biochemical (digestive enzymes) ‘factors, contributing to greater stability in the coloring potential of these pigments (Zhao et al., 2017; Carrillo et al., 2020; Fei et al., 2021; Vidana Gamage et al., 2021). In potato, all six most common anthocyanidins have been found in the tubers, giving them a large complexity of colors and nuances (Figure 1).

#### 3.2. Genetic regulation of anthocyanin biosynthesis

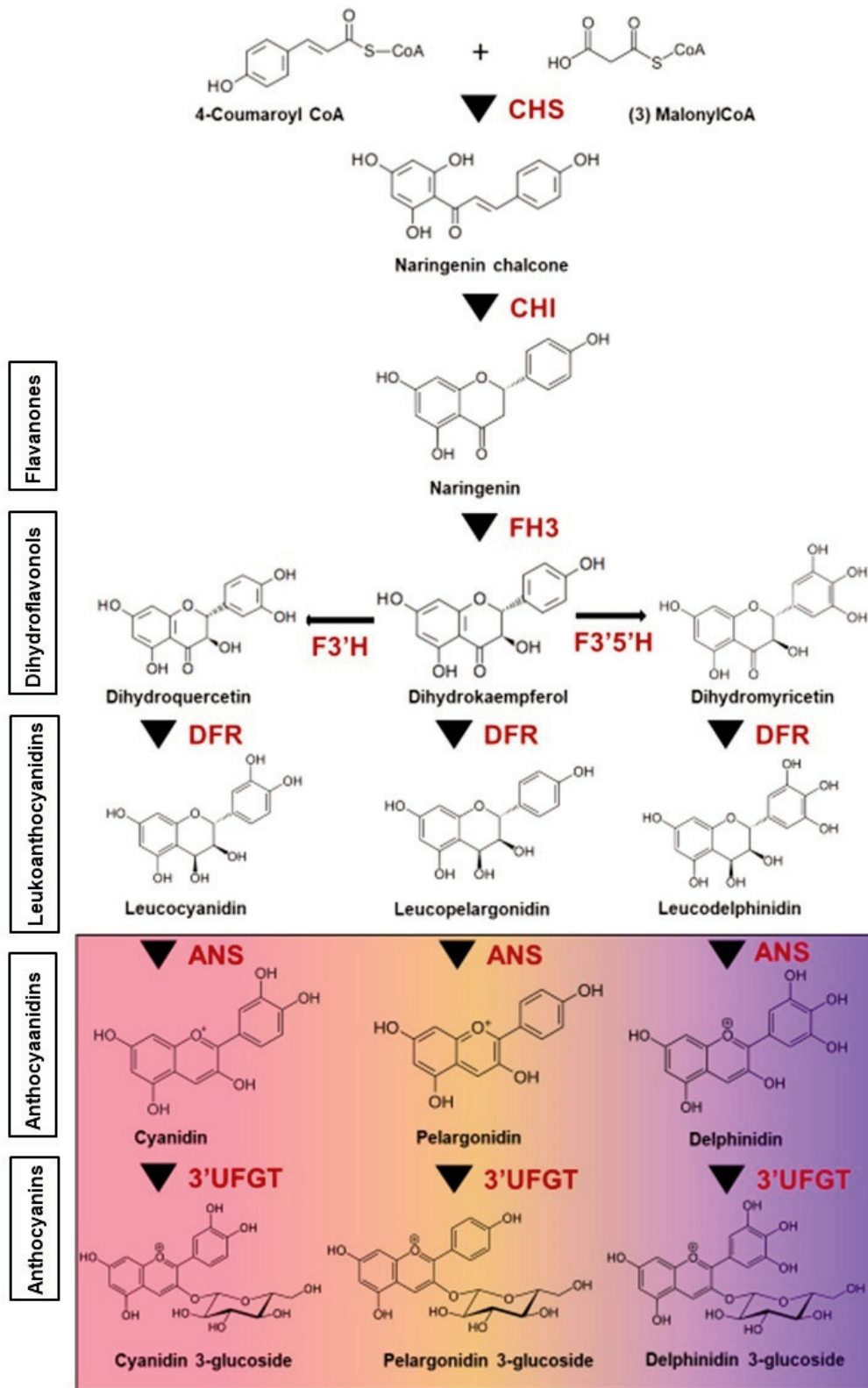
The biosynthetic pathway of anthocyanins is well characterized and conserved in many plant species, including *S. tuberosum* and its wild relatives (D’Amelia et al., 2018; Strygina et al., 2019). The enzymes involved in the biosynthesis of anthocyanidins are mainly localized in the endoplasmic reticulum, organized into a multi-enzyme complex named flavonoid metabolon (Fujino et al., 2018). The biosynthetic pathway can be divided into two sections, the basic flavonoid upstream pathway, which includes early biosynthetic genes (EBGs), and the specific anthocyanin downstream branch, which includes late biosynthetic genes (LBGs) ‘(Lachman et al., 2009). It begins with the synthesis of naringenin chalcone starting from 4-coumaroyl-CoA and malonyl-CoA mediated by the enzyme chalcone synthase (CHS) ‘(Figure 2). Naringenin chalcone is then isomerized by chalcone isomerase (CHI) ‘into naringenin. Flavanone 3-hydroxylase (FH3) ‘enzyme converts naringenin into dihydrokaempferol



**Figure 1.** Representative image of the principal anthocyanidin patterns in red and blue pigmented potatoes. The presence of all six most common anthocyanidins gives to potato tubers a unique and wide range of colors characteristic of both skin and flesh (Lachman et al., 2009; Burmeister et al., 2011; De Masi et al., 2020).

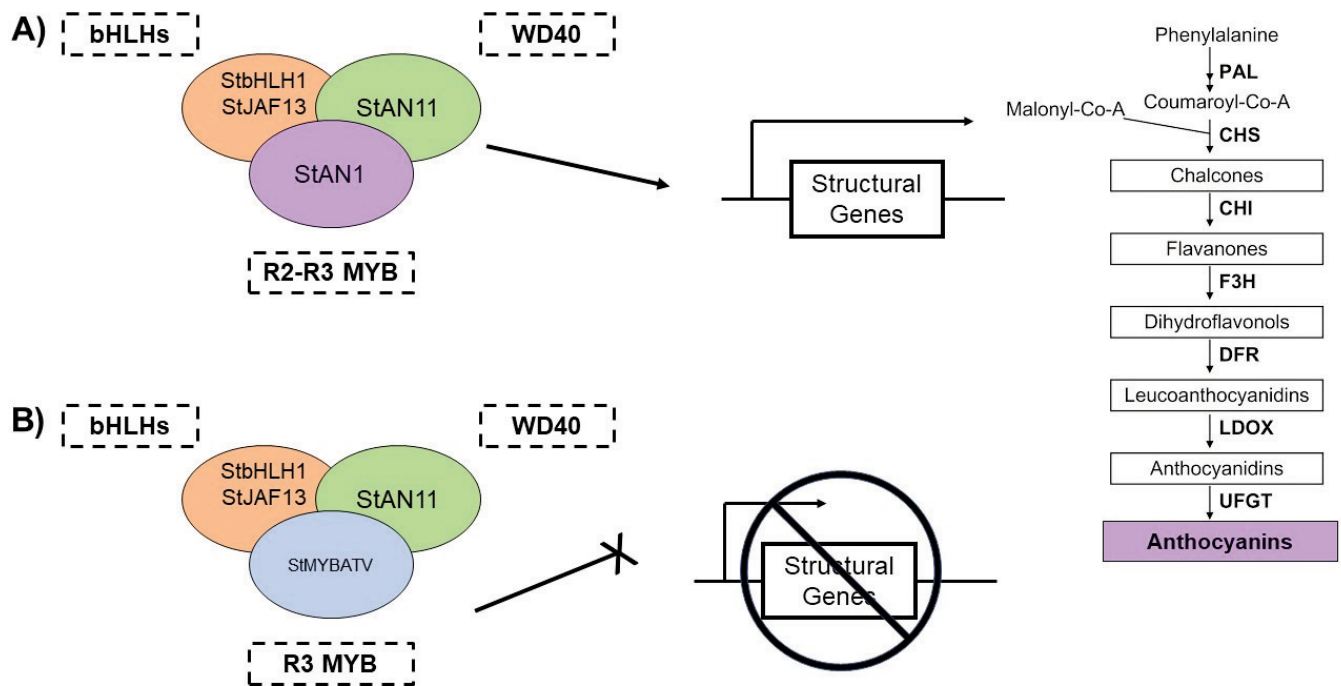
which can be hydroxylated by flavonoid 3'-hydroxylase (F3'H) 'or flavonoid 3'-5'-hydroxylase (F3'5'H) 'in others two dihydroflavonols, respectively dihydroquercetin or dihydromyricetin. Subsequently, the three dihydroflavonols are converted to colorless leucoanthocyanidins by dihydroflavonol 4-reductase (DFR) 'and later to anthocyanidins colored by anthocyanidin synthase (ANS). Finally, the sugar moieties are attached to anthocyanidins by various members of the glycosyltransferase family, such as the flavonoid 3-O-glucosyltransferase (UFGT), and can be further acetylated with acylaromatic groups by the acyltransferases. CHS is therefore the initial key enzyme for the biosynthesis of flavonoids. F3'H and F3'5'H are the main enzymes responsible for the diversification of anthocyanins, determined by the hydroxylation of the catechol, with consequent variation in color (Liu et al., 2018). A positive correlation between LBG expression levels and anthocyanin content has been observed (André et al., 2009, Tengkun et al., 2019). In fact, in potato peel with high anthocyanin pigmentation, the genes *StF3'H*, *StF3'5'H*, *StDRF*, *StANS*, and *StUFGT* were highly expressed (Jung et al., 2009; André et al., 2009; Tengkun et al., 2019). Furthermore, *StDRF* was found to be highly expressed in the pulp of red and purple cultivars Colorado Rose, Mountain Rose, Purple Majesty, and Russet Nugget, indicating a strong correlation between this gene and pigmentation (Stushnoff et al., 2010).

The structural genes of the anthocyanin biosynthetic pathway are under the control of a regulatory complex, called MYB-bHLH-WD40 (MBW). The expression of the *MYB* genes, mainly belonging to the R2R3-MYB subfamily, and of the basic helix loop helix *bHLH* genes, belonging to subdivision IIIf (Heim et al., 2003), is largely specific for pigmented tissues. By contrast, the expression of the genes encoding the WD40 factors, involved in the stabilization of MBW complexes, is similar between pigmented and non-pigmented tissues (Koes et al., 2005). The R2R3-MYB transcription factors consist of a conserved domain (R2R3) 'binding DNA and are the key element for the tissue-specific accumulation of anthocyanins. In potatoes, the gene that codes for an activator of anthocyanins of the R2R3-MYB



**Figure 2.** Schematic representation of the anthocyanin biosynthetic pathway. In red are the structural genes regulating each step of the pathway: *CHS*, chalcone synthase; *CHI*, chalcone isomerase; *F3H*, flavanone 3-hydroxylase; *F3'H*, flavonoid 3'-hydroxylase; *F3'5'H*, flavonoid 3',5'-hydroxylase; *DFR*, dihydroflavonol 4-reductase; *ANS*, anthocyanidin synthase; *3'UGFT*, flavonoid 3-O-glucosyltransferase. Modified from Mekapogu et al., 2020.

class is called *StANI* (Jung et al., 2009). Among the differently pigmented varieties, there is a variation in the number of repeats in a 10 amino acid motif at the C-terminus of *StANI* (Strygina et al. 2019). BHLH proteins are the second largest class of transcription factors (Duan et al., 2021). Generally, the first 200 amino acids of this protein interact with the MYB factors, while the next 200 amino acids interact with the WD40 protein (D’Amelia et al., 2014). The bHLH domains are involved in the formation of homo- or heterodimers with other bHLH proteins; this appears to be a prerequisite for the recognition of binding sites on DNA, contributing to binding specificity (Montefiori et al., 2015). In Solanaceae, there are two main clades involved in the regulation of anthocyanin biosynthesis, which are orthologues of the *PhANI* and *PhJAF13* genes identified in Petunia. In potato, the *StbHLH1* gene, ortholog of *PhANI*, is widely expressed in red and purple tubers (Payyavula et al., 2013). *StbHLH1* expression alone is not associated with anthocyanin biosynthesis in the tuber and co-expression of the *StANI* gene is required (Liu et al., 2015). WD40 proteins provide a stable platform for bHLH and MYB proteins during the establishment of the MBW complex. In Solanaceae, in addition to these transcriptional factors activating anthocyanins, MYB repressors are present, which reduce the biosynthesis of anthocyanins (Figure 3). These repressors are divided into two categories: R2R3-MYB repressors, containing a typical motif at the C-terminus, change the function of the MBW complex from activator to repressor of downstream gene transcription; R3-MYB repressors, on the other hand, do not have a repressor motif and are unable to act directly on the target genes (Liu et al., 2018). They show “passive suppression”, which means that they compete with MYB activators for interaction with bHLH, thus reducing the pool of MBW complexes that are able to bind the promoters of genes for anthocyanin



biosynthesis. An R3-MYB repressor, named *StMYBATV*, has been identified in potatoes (D’Amelia et al., 2020).

**Figure 3.** A simplified model outlining the regulatory mechanism of potato transcription factors involved in the MBW complex, MYB, bHLH, and WD40. They modulate the expression of structural genes of the anthocyanin biosynthetic pathway. (A) ‘Active regulation of anthocyanin biosynthesis. (B)

‘Repressive regulation of anthocyanin biosynthesis. MYB repressors compete with MYB activators for *StbHLH1/StJAF13*. “→” means activation and “—X” means repression.

#### 4. Anthocyanins: an important resource for food and drugs industry

Due to their role in human health, plant researchers are becoming more addressed into both the identification of health-promoting anthocyanins among the most worldwide cultivated crops (such as *Z. mays*, *S. tuberosum*, and *Ipomea batatas*) ‘and the validation of their functional activity against several non-communicable human diseases (Speer et al., 2020). Numerous studies tested the biological and pharmacological properties of anthocyanins, including antioxidant, anticancer, antidiabetic, antimicrobial, antiatherosclerotic, and neuroprotective activity (Swallah et al., 2020). Further, their intake through the consumption of foods rich in flavonoids inhibits pro-inflammatory cytokines induced by hydrogen peroxide, enhances antioxidant enzyme activities and improves the redox balance thanks to their high scavenging activities (Zhang et al., 2016; Pekas et al., 2021). Given that the potato is one of the most consumed vegetables worldwide and that its anthocyanins show post-biosynthetic decorations (e.g. acylations), the validation of their antioxidative and anti-tumoral activities is very impactful. In a recent paper by De Masi et al. (2020), phenolic compounds extracted from anthocyanin-rich potato varieties showed antiproliferative and apoptotic effects in hematological cancer cell lines. Similarly, the results offered by Charepalli et al. (2015) ‘suggested that treatment with anthocyanin-rich extracts from potato tubers of the cultivar Purple Majesty had protective effect against colon tumorigenesis. The beneficial activities of anthocyanins are also widely employed in animal feed as dietary supplementation (Hosoda et al. 2012; Choi et al., 2013).

Anthocyanins are also valuable compounds as antioxidants and antimicrobial for food storage and industry. Indeed, they play an important role in extending postharvest shelf-life (Zhang et al., 2015; Petric et al., 2018; Chen et al., 2020). What is more, there is a growing demand for the use of preservatives derived from natural products. The preservative property of anthocyanins is mainly due to their antioxidant potential which can positively boost food storage with different model of action. For example, anthocyanins reduce lipid autoxidation, which is a major cause of shelf-life decreasing and rotting (Shahidi and Ambigaipalan 2015). For this reason, anthocyanins extracts are attractive for use in meat products because they are compatible in terms of solubility and they delay lipid and/or protein oxidation, and reduce discoloration (Prommachart et al, 2020; Aksu and Turan, 2021). The antioxidant action of anthocyanins to extend shelf-life is also related to their ability to reduce the susceptibility of fresh fruits and vegetables to specific postharvest pathogens. As illustrated by Zhang et al. (2015), high hydroxylated anthocyanins are able to block the development of grey mold (caused by *Botrytis cinerea*) ‘perturbing the dynamics of the ROS burst during infection in tomato fruits. Potato anthocyanin-rich aqueous extracts are effective fungicide against different micromycetes and they could be applied in soft drink formulation, showing suitable profiles in the sensory and shelf-life assessments (Sampaio et al., 2021). Anthocyanins can also provide antimicrobial activity against several microorganisms since they can bind and inactivate proteins and may complex with bacterial cell walls (Hintz et al. 2015). Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by a colour change and different absorbance spectra. The red colored oxonium form predominates at pH 1.0, the colorless hemiketal form at pH 4.5 and at pH>8 the colour change from blue to yellow, indicating that the anthocyanin structure is in the form of yellow chalcone under alkaline conditions (Castañeda-Ovando et al., 2009). For this reason, anthocyanins can be used in monitoring food quality with intelligent packaging. Indeed, pH indicator and antimicrobial cellulose nanofibre packaging film has been developed based on cellulose nanofibres as film matrix and anthocyanins from purple potato as a natural dye and as an antimicrobial agent (Chen et al., 2020). Anthocyanins are also used as food colorants. Food additive E163 is one of the commercial additives derived from fruit anthocyanin such as grape skin, and it is used in purple-colored jam, confectionaries, and beverages (Khoo et al., 2017). The application of anthocyanins as a natural colorant is not widely spread because its production is strictly

dependent on the seasonality of the primary source, and because anthocyanins showed low stability and weak tinctorial strength. Since high levels of glycosylation and acylation may enhance the stability and antioxidant properties, sources of more complex forms of anthocyanins are being identified. In this regard, genetic studies and innovative biotechnological applications are expected to play a key role to both identifying and enhancing anthocyanin variability, as reported in Table 1.

Species	Tissue type	Type of approach	References
<i>Dendrobium</i> Sabin Blue	Protocorm-like bodies (PBLs)	Treatment with yeast extract	Chin et al., 2021
<i>Raphanus sativus</i>	Adventitious root culture	Supplement of auxins	Betsui et al., 2004
<i>Rosa hybrida</i>	Embryogenic calli	Heterologous expression of <i>F3'5'H</i>	Katsumoto et al., 2008
<i>Solanum nigrum</i>	Shoot culture	Overexpression of <i>AtPAP1</i>	Chhon et al., 2020
<i>Ipomea batatas</i>	Calli culture	Overexpression of <i>IbMYB1</i> TF	Mano et al., 2007
<i>Nicotiana</i> spp.	Leafs	Transient expression of multi-genic construct	Fresquet-Corrales et al., 2017
<i>Vitis vinifera</i>	Cell suspension culture	Treatment with Methyl Jasmonate	Qu et al., 2011
<i>Ginkgo biloba</i>	Cell suspension culture	Treatment with fungal endophytes	Hao et al., 2010
<i>Antirrhinum majus</i>	Hairy root culture	Ectopic expression of <i>AmRosea1</i>	Piao et al., 2021
<i>Melissa officinalis</i>	Shoot cultures	Treatment with ozone	Tonelli et al., 2015

**Table 1.** Promising biotechnological strategies used for the improvement of anthocyanins quality and quantity in plant cell and organ cultures.

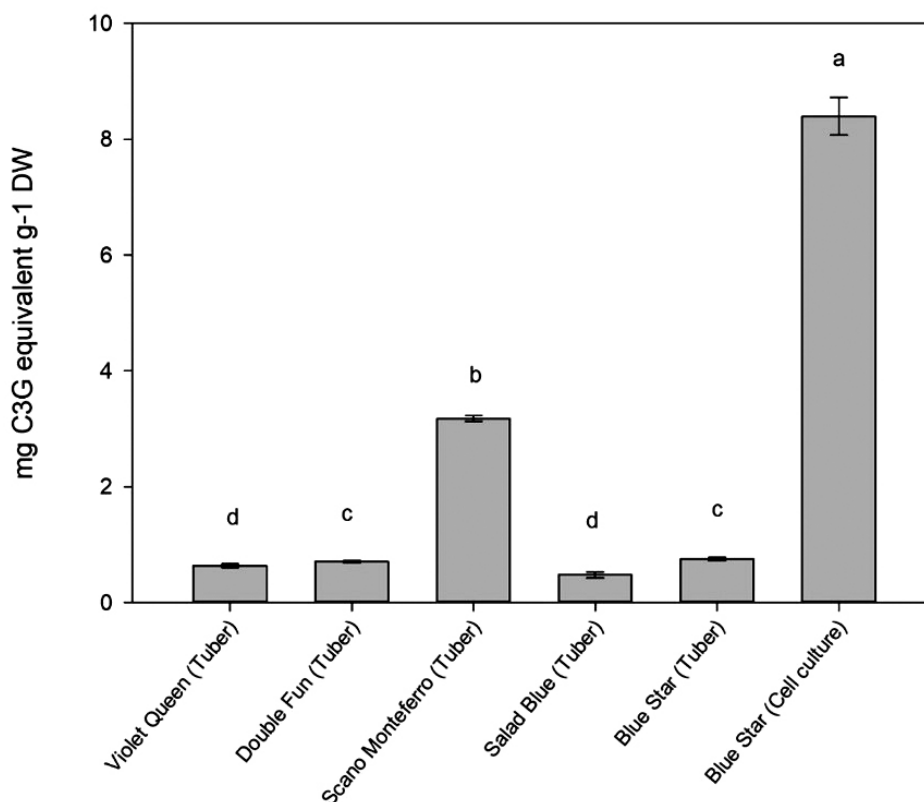
### 5. Plant biofactories for anthocyanin production and the advantages of molecular farming in potato cell cultures

The global anthocyanin market size was worth USD 318 million in 2020 and is expected to grow at a compound annual rate of 4.6%, to reach USD 388 million in 2026 (Market Data Forecast, 2021). The large-scale production of anthocyanin pigments is mainly based on the extraction from food industry waste, such as grape skin, red cabbage, sweet potato, and berries (Rodriguez-Amaya, 2016). The composition of anthocyanins from these natural resources varies qualitatively and quantitatively, according to the genetic background, the region of origin, and the growing season of the plant (Timmers et al., 2017). This has prompted manufacturers to identify alternative sources of food colors and natural compounds with cosmetic and medicinal properties. The production of these natural compounds could be carried out economically, effectively, and sustainably through the strategic application of biotechnologies. The use of plant cell cultures in bioreactors may provide an affordable and versatile strategy for anthocyanin production. They have already been successfully used for the production of alkaloids, flavonoids, and terpenes (Simões et al., 2012). Our unpublished results provided evidence that anthocyanin content in potato cell cultures (variety Blue Star) 'is much higher than that of tubers of other polyphenol-rich potato varieties (Figure 4). Based on a simple calculation considering the average concentration of anthocyanin-rich cultivars, it is important to underly that the average yield of Andean cultivar is approximately 44 t/ha (Pazderu et al., 2015), therefore the total anthocyanin concentration in purple potatoes is approximately 44-66 kg/ha. This production has severe limitations, such as the single harvest per year and the massive exploitation of productive farmland, while bioreactor systems with suspension cell cultures, besides the higher yield in anthocyanin content, are cost-effective, time- and space-saving.

Suspension cell cultures are an *in vitro* cultivation system that uses a friable callus inoculum, characterized by disorganized parenchymal tissues, to obtain single cells or small aggregates. The interest in this system arises from various advantages, such as independence from the environment and climatic conditions and the guarantee of continuous supplies, in terms of quantity and quality, of specialized metabolites. Also, potato cell cultures offer a controllable model system that allows to prevent physio-



**Figure 4.** Monomeric anthocyanin content (cyanidin-3-O-glucoside) in extracts from color-fleshed potato varieties and cell culture of Blue Star. Values are the means of three replicates; bars with different letters indicate significant differences according to Tukey's test at  $P \leq 0.001$ .



logical and biochemical processes caused by stress factors, as enzymatic browning reactions (Dörnenburg et al., 1997). Cell cultures are maintained in a suspension state thanks to the use of plant growth regulators, such as auxins and cytokines. In potato, several protocols have been developed for cell cultures with different hormone combinations (Table 2). Callus can be initiated from almost any part of the potato plant, as tubers, leaf sections and internodes. Once the callus has been induced, it is necessary to transfer it to a stirred liquid substrate, usually dispensed in flasks placed on orbital moving platforms and subsequently in reactors. The liquid state of the substrate facilitates the gaseous and nutritional exchanges between the cells and the culture medium, while the agitation allows the aeration and prevents the phenomena of cell aggregation (Mustafa et al., 2011). Nutrient and precursor feeding are used to improve the yield of secondary metabolites production, since cultivated cells have the capacity for biotransformation of supplied compounds into high-value compounds. This can occur through different reactions as hydroxylation, methylation, oxidation of hydroxyl group, glycosyl conjugation, acylation, and hydrolysis, catalyzed by plant enzymes (Murthy et al., 2014). Elicitors may be key factors for the stimulation of biosynthesis of specialized metabolites during *in vitro* culture conditions. At the bioreactor scale, elicitors must be chosen very precisely in terms of optimum concentration and combination for gaining maximum anthocyanin content and yield, requiring a long preliminary process (D'Amelia et al., 2021). Among elicitors, abiotic stresses, such as UV-irradiation, drought, wounding, nutrient deficiencies, and low temperatures, often increase the accumulation of anthocyanins (Ramakrishna et al., 2011; Cirillo et al., 2021). Biotic elicitors such as chitosan, chitin, polysaccharides, and oligosaccharides are also reported to trigger the anthocyanin biosynthetic pathway. They alter biochemical and physiological processes because they induce plant defense responses similar to pathogen invasion (Chandran et al., 2020). Previous publications showed that potato cell cultures can be gradually acclimated to various abiotic elicitors such as salt stress (Sabbah et al., 1990) and frost (Van Swaaij et

al., 1987). However, to the authors' knowledge, the use of elicitors in potato has been scarcely investigated for the production of anthocyanins in cell cultures. Previous research focusing on *in planta* anthocyanin production after cold (D'Amelia et al., 2018), in response to *Streptomyces scabiei* infection (Tai et al., 2013), and after the use of inorganic elicitors as sodium selenite (Lei et al., 2014) 'may be the starting point for further studies on massive anthocyanin production in potato cell cultures. Currently, the production of anthocyanins in *in vitro* cultures has been reported from various plant species, including *Daucus carota*, *Rosa hybrida*, and *Vitis vinifera* (Belwal et al., 2020).

Explant	Medium	Auxin	Cytokinin	Reference
Leaf	MS	5 mg L <sup>-1</sup> NAA		Van Swaaij et al., 1987
	MS	3 mg L <sup>-1</sup> 2,4-D	1 mg L <sup>-1</sup> K	Sapko et al., 2011
	MS	2 mg L <sup>-1</sup> 2,4 D	0.25 mg L <sup>-1</sup> K	Leone et al., 1994
Leaf rachis	MS	5 mg L <sup>-1</sup> 2,4 D	0.5 mg L <sup>-1</sup> K	Sabbah et al., 1990
Tubers	MS	1 mg L <sup>-1</sup> 2,4-D		Dörnerburg et al., 1997

**Table 2.** Explant sources and media used to initiate suspension cultures of potato.

## 6. Conclusions and future perspectives

Apart from the extraction of anthocyanins directly from natural sources, *in vitro* cultures represent a smart and gainful alternative for anthocyanin production. In this scenario, the application of novel techniques as genome editing represents an innovative strategy for the targeted and punctual modifications of cell genes and regulators. Among the different genome editing tools, CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats-associated protein 9) 'has a huge potential and could provide tailor-made anthocyanins-rich cells, enhancing the quantity as well as the antioxidant capacity. Along with genome editing approaches, the advancement in sequencing technologies (Next-Generation Sequencing) 'can be fruitful in the identification of novel gene variants of the anthocyanin biosynthetic pathway.

## References

- Aksu, M.İ., Turan, E. (2021) 'Effects of lyophilized black carrot (*Daucus carota* L.) water extract on the shelf life, physico-chemical and microbiological quality of high-oxygen modified atmosphere packaged (HiOx-MAP) 'ground beef' *Journal of Food Science and Technology*, pp. 1-11. doi: [10.1007/s13197-021-05044-1](https://doi.org/10.1007/s13197-021-05044-1)
- Akula, R., Ravishankar, G.A. (2011) 'Influence of abiotic stress signals on secondary metabolites in plants' *Plant Signaling & Behavior*, 6(11), pp. 1720-1731. doi: [10.4161/psb.6.11.17613](https://doi.org/10.4161/psb.6.11.17613)
- Andersen, Ø.M., Jordheim, M. (2005) 'The anthocyanins' In *Flavonoids: Chemistry, Biochemistry and Applications*. CRC Press, pp. 471–551.
- André, C.M., Oufir, M., Guignard, C., Hoffmann, L., Hausman, J.F., Evers, D., Larondelle, Y. (2007) 'Antioxidant profiling of native Andean potato tubers (*Solanum tuberosum* L.) 'reveals cultivars with high levels of β-carotene, α-tocopherol, chlorogenic acid, and petanin' *Journal of Agricultural and Food Chemistry*, 55(26), pp. 10839-10849. doi: [10.1021/jf0726583](https://doi.org/10.1021/jf0726583)
- André, C.M., Schafleitner, R., Legay, S., Lefèvre, I., Aliaga, C.A., Nomberto, G. (2009) 'Gene expression changes related to the production of phenolic compounds in potato tubers grown under drought stress' *Phytochemistry*, 70, pp. 1107–1116. doi: [10.1016/j.phytochem.2009.07.008](https://doi.org/10.1016/j.phytochem.2009.07.008)
- Appelhaagen, I., Wulff-Vester, A.K., Wendell, M., Hvoslef-Eide, A.K., Russell, J., Oertel, A., Matros, A. (2018) 'Colour bio-factories: Towards scale-up production of anthocyanins in plant cell cultures' *Metabolic Engineering*, 48, pp. 218-232. doi: [10.1016/j.ymben.2018.06.004](https://doi.org/10.1016/j.ymben.2018.06.004)
- Aversano, R., Contaldi, F., Ercolano, M.R., Grosso, V., Iorizzo, M., Tatino, F., Carputo, D. (2015) 'The *Solanum commersonii* genome sequence provides insights into adaptation to stress conditions and

- genome evolution of wild potato relatives' *The Plant Cell*, 27(4), pp. 954-968. doi: [10.1105/tpc.114.13595](https://doi.org/10.1105/tpc.114.13595)
- Barrell, P.J., Meiyalaghan, S., Jacobs, J.M., Conner, A.J. (2013) 'Applications of biotechnology and genomics in potato improvement' *Plant Biotechnology Journal*, 11(8), pp. 907-920. doi: [10.1111/pbi.12099](https://doi.org/10.1111/pbi.12099)
- Belwal, T., Singh, G., Jeandet, P., Pandey, A., Giri, L., Ramola, S., Luo, Z. (2020) 'Anthocyanins, multi-functional natural products of industrial relevance: Recent biotechnological advances' *Biotechnology Advances*, 107600. doi: [10.1016/j.biotechadv.2020.107600](https://doi.org/10.1016/j.biotechadv.2020.107600)
- Betsui, F., Tanaka-Nishikawa, N., Shimomura, K. (2004) 'Anthocyanin production in adventitious root cultures of *Raphanus sativus* L. cv. Peking Koushin' *Plant Biotechnology*, 21(5), pp. 387-391. doi: [10.5511/plantbiotechnology.21.387](https://doi.org/10.5511/plantbiotechnology.21.387)
- Bhaskar, R., Xavier, L.S.E., Udayakumaran, G., Kumar, D.S., Venkatesh, R., Nagella, P. (2021) 'Biotic elicitors: A boon for the in-vitro production of plant secondary metabolites' *Plant Cell, Tissue and Organ Culture (PCTOC)*, pp. 1-18. doi: [10.1007/s11240-021-02131-1](https://doi.org/10.1007/s11240-021-02131-1)
- Bontpart, T., Cheynier, V., Ageorges, A., Terrier, N. (2015) 'BAHD or SCPL acyltransferase? What a dilemma for acylation in the world of plant phenolic compounds' *New Phytologist*, 208(3), pp. 695-707. doi: [10.1111/nph.13498](https://doi.org/10.1111/nph.13498)
- Burmeister, A., Bondiek, S., Apel, L., Kühne, C., Hillebrand, S., Fleischmann, P. (2011) 'Comparison of carotenoid and anthocyanin profiles of raw and boiled *Solanum tuberosum* and *Solanum phureja* tubers' *Journal of Food Composition and Analysis*, 24(6), pp. 865-872. doi: [10.1016/j.jfca.2011.03.006](https://doi.org/10.1016/j.jfca.2011.03.006)
- Carrillo, C., Kamiloglu, S., Grootaert, C., Van Camp, J., Hendrickx, M. (2020) 'Co-Ingestion of Black Carrot and Strawberry. Effects on anthocyanin stability, Bioaccessibility and uptake' *Foods*, 9(11), pp. 1595. doi: [10.3390/foods9111595](https://doi.org/10.3390/foods9111595)
- Castañeda-Ovando, A., de Lourdes Pacheco-Hernández, M., Páez-Hernández, M.E., Rodríguez, J.A., Galán-Vidal, C.A. (2009) 'Chemical studies of anthocyanins: A review' *Food Chemistry*, 113(4), pp. 859-871. doi: [10.1016/j.foodchem.2019.125373](https://doi.org/10.1016/j.foodchem.2019.125373)
- Chandran, H., Meena, M., Barupal, T., Sharma, K. (2020) 'Plant tissue culture as a perpetual source for production of industrially important bioactive compounds' *Biotechnology Reports*, 26, e00450. doi: [10.1016/j.btre.2020.e00450](https://doi.org/10.1016/j.btre.2020.e00450)
- Charepalli, V., Reddivari, L., Radhakrishnan, S., Vadde, R., Agarwal, R., Vanamala, J.K. (2015) 'Anthocyanin-containing purple-fleshed potatoes suppress colon tumorigenesis via elimination of colon cancer stem cells' *The Journal of Nutritional Biochemistry*, 26(12), pp. 1641-1649. doi: [10.1016/j.jnutbio.2015.08.005](https://doi.org/10.1016/j.jnutbio.2015.08.005)
- Chen, S., Wu, M., Lu, P., Gao, L. (2020) 'Development of pH indicator and antimicrobial cellulose nanofibre packaging film based on purple sweet potato anthocyanin and oregano essential oil' *International Journal of Biological Macromolecules*, 149, pp. 271-280. doi: [10.1016/j.ijbiomac.2020.01.231](https://doi.org/10.1016/j.ijbiomac.2020.01.231)
- Chhon, S., Jeon, J., Kim, J., Park, S.U. (2020) 'Accumulation of anthocyanins through overexpression of AtPAP1 in *Solanum nigrum* Lin. (Black Nightshade)' *Biomolecules*, 10(2), pp. 277. doi: [10.3390/biom10020277](https://doi.org/10.3390/biom10020277)
- Chin, C.K., Stanly, C., Muniandy, A., Chew, B.Y., Subramaniam, S. (2021) 'Protocorm-like bodies (PLBs) 'of *Dendrobium Sabin Blue*: a novel source for in vitro production of dendrobine and anthocyanin' *In Vitro Cellular and Developmental Biology-Plant*. doi: [10.1007/s11627-021-10159-3](https://doi.org/10.1007/s11627-021-10159-3)
- Choi, M.K., Park, S.J., Eom, S.H., Kang, M.H. (2013) 'Anti-diabetic and hypolipidemic effects of purple-fleshed potato in streptozotocin-induced diabetic rats' *Food Science and Biotechnology*, 22(5), pp. 1-6. doi: [10.1007/s10068-013-0231-5](https://doi.org/10.1007/s10068-013-0231-5)
- Chowdhary, P., Gupta, A., Gnansounou, E., Pandey, A., Chaturvedi, P. (2021) 'Current trends and pos-

- sibilities for exploitation of grape pomace as a potential source for value addition' *Environmental Pollution*, 278, 116796. doi: [10.1016/j.envpol.2021.116796](https://doi.org/10.1016/j.envpol.2021.116796)
- Cirillo, V., D'Amelia, V., Esposito, M., Amitrano, C., Carillo, P., Carputo, D., Maggio, A. (2021) 'Anthocyanins are key regulators of drought stress tolerance in tobacco' *Biology (Basel)*, 10(2), 139. doi: [10.3390/biology10020139](https://doi.org/10.3390/biology10020139)
- Cress, B.F., Leitz, Q.D., Kim, D.C., Amore, T.D., Suzuki, J.Y., Linhardt, R.J., Koffas, M.A. (2017) 'CRISPRi-mediated metabolic engineering of E. coli for O-methylated anthocyanin production' *Microbial Cell Factories*, 16(1), pp. 1-14. doi: [10.1186/s12934-016-0623-3](https://doi.org/10.1186/s12934-016-0623-3)
- Cui, L., Ni, X., Ji, Q., Teng, X., Yang, Y., Wu, C., Kai, G. (2015) 'Co-overexpression of geraniol-10-hydroxylase and strictosidine synthase improves anti-cancer drug camptothecin accumulation in *Ophiorrhiza pumila*' *Scientific Reports*, 5(1), pp. 1-9. doi: [doi.org/10.1038/srep08227](https://doi.org/10.1038/srep08227)
- Cui, T., Bai, J., Zhang, J., Zhang, J., Wang, D. (2014) 'Transcriptional expression of seven key genes involved in steroidal glycoalkaloid biosynthesis in potato microtubers' *New Zealand Journal of Crop and Horticultural Science*, 42(2), pp. 118-126. doi: [10.1080/01140671.2013.870219](https://doi.org/10.1080/01140671.2013.870219)
- D'Amelia, V., Aversano, R., Batelli, G., Caruso, I., Moreno, M.C., Castro-Sanz, A.B. (2014) 'High AN1 variability and interaction with basic helix-loop-helix co-factors related to anthocyanin biosynthesis in potato leaves' *Plant Journal*, 80, pp. 527-540. doi: [10.1111/tpj.12653](https://doi.org/10.1111/tpj.12653)
- D'Amelia, V., Aversano, R., Chiaiese, P., Carputo, D. (2018) 'The antioxidant properties of plant flavonoids: their exploitation by molecular plant breeding' *Phytochemistry Reviews*, 17, pp. 611-625. doi: [10.1007/s11101-018-9568-y](https://doi.org/10.1007/s11101-018-9568-y)
- D'Amelia, V., Docimo, T., Crocoll, C., Rigano, M.M. (2021) 'Specialized metabolites and valuable molecules in crop and medicinal plants: the evolution of their use and strategies for their production' *Genes*, 12(6), 936. doi: [10.3390/genes12060936](https://doi.org/10.3390/genes12060936)
- D'Amelia, V., Villano, C., Batelli, G., Çobanoğlu, O., Carucci, F., Melito, S., Chessa, M., Chiaiese, P., Aversano, R., Carputo, D. (2020) 'Genetic and epigenetic dynamics affecting anthocyanin biosynthesis in potato cell cultures' *Plant Science*, 298, 110597. doi: [10.1016/j.plantsci.2020.110597](https://doi.org/10.1016/j.plantsci.2020.110597)
- D'Amelia, V., Aversano, R., Ruggiero, A., Batelli, G., Appelhagen, I., Dinacci, C., Carputo, D. (2018) 'Subfunctionalization of duplicate MYB genes in *Solanum commersonii* generated the cold-induced ScAN2 and the anthocyanin regulator ScAN1' *Plant, Cell & Environment*, 41(5), pp. 1038-1051. doi: [10.1111/pce.12966](https://doi.org/10.1111/pce.12966)
- Dangol, S.D., Barakate, A., Stephens, J., Çalıskan, M.E., Bakhsh, A. (2019) 'Genome editing of potato using CRISPR technologies: current development and future prospective' *Plant Cell, Tissue and Organ Culture (PCTOC)*, 139(2), pp. 403-416. doi: [10.1007/s11240-019-01662-y](https://doi.org/10.1007/s11240-019-01662-y)
- Davies, K. M., Albert, N. W., Schwinn, K. E. (2012) 'From landing lights to mimicry: the molecular regulation of flower colouration and mechanisms for pigmentation patterning' *Functional Plant Biology*, 39(8), pp. 619-638. doi: [10.1071/FP12195](https://doi.org/10.1071/FP12195)
- Davies, K.M., Deroles, S.C. (2014) 'Prospects for the use of plant cell cultures in food biotechnology' *Current Opinion in Biotechnology*, 26, pp. 133-140. doi: [10.1016/j.copbio.2013.12.010](https://doi.org/10.1016/j.copbio.2013.12.010)
- De Jong, W., Eannetta, N., De Jong, D., Bodis, M. (2004) 'Candidate gene analysis of anthocyanin pigmentation loci in the Solanaceae' *Theoretical and Applied Genetics*, 108(3), pp. 423-32. doi: [10.1007/s00122-003-1455-1](https://doi.org/10.1007/s00122-003-1455-1)
- De Masi, L., Bontempo, P., Rigano, D., Stiuso, P., Carafa, V., Nebbioso, A., Piacente, S., Montoro, P., Aversano, R., D'Amelia, V., Carputo, D., Altucci, L. (2020) 'Comparative phytochemical characterization, genetic profile, and antiproliferative activity of polyphenol-rich extracts from pigmented tubers of different *Solanum tuberosum* varieties' *Molecules*, 25(1), 233. doi: [10.3390/molecules25010233](https://doi.org/10.3390/molecules25010233)
- Dörnenburg, H., Knorr, D. (1997) 'Evaluation of elicitor-and high-pressure-induced enzymatic browning utilizing potato (*Solanum tuberosum*) 'suspension cultures as a model system for plant tissues' *Journal of Agricultural and Food Chemistry*, 45(10), pp. 4173-4177. doi: [10.1021/jf9701603](https://doi.org/10.1021/jf9701603)

- Duan, Z., Tian, S., Yang, G., Wei, M., Li, J., Yang, F. (2021) ‘The basic helix-loop-helix transcription factor SmbHLH1 represses anthocyanin biosynthesis in eggplant’ *Frontiers in Plant Science*, 2435. doi: [10.3389/fpls.2021.757936](https://doi.org/10.3389/fpls.2021.757936)
- Dudnik, A., Almeida, A.F., Andrade, R., Avila, B., Bañados, P., Barbay, D., Bassard, J.E. (2018) ‘BacHBerry: bacterial hosts for production of bioactive phenolics from berry fruits’ *Phytochemistry Reviews*, 17, pp. 291-326. doi: [10.1007/s11101-017-9532-2](https://doi.org/10.1007/s11101-017-9532-2)
- Fei, P., Zeng, F., Zheng, S., Chen, Q., Hu, Y., Cai, J. (2021) ‘Acylation of blueberry anthocyanins with maleic acid: Improvement of the stability and its application potential in intelligent color indicator packing materials’ *Dyes and Pigments*, 184: 108852. doi: [10.1016/j.dyepig.2020.108852](https://doi.org/10.1016/j.dyepig.2020.108852)
- Fresquet-Corrales, S., Roque, E., Sarrión-Perdigones, A., Rochina, M., López-Gresa, M.P., Díaz-Mula, H.M., Cañas, L.A. (2017) ‘Metabolic engineering to simultaneously activate anthocyanin and proanthocyanidin biosynthetic pathways in *Nicotiana* spp.’ *PloS one*, 12(9), e0184839. doi: [10.1371/journal.pone.0184839](https://doi.org/10.1371/journal.pone.0184839)
- Fujino, N., Tenma, N., Waki, T., Ito, K., Komatsuzaki, Y., Sugiyama, K., Yamazaki, T., Yoshida, S., Hatayama, M., Yamashita, S., Tanaka, Y., Motohashi, R., Denessiouk, K., Takahashi, S. and Nakayama, T. (2018) ‘Physical interactions among flavonoid enzymes in snapdragon and torenia reveal the diversity in the flavonoid metabolon organization of different plant species’ *The Plant Journal*, 94, pp. 372-392. doi: [10.1111/tpj.13864](https://doi.org/10.1111/tpj.13864)
- Georgiev, V., Slavov, A., Vasileva, I., Pavlov, A. (2018) ‘Plant cell culture as emerging technology for production of active cosmetic ingredients’ *Engineering in Life Sciences*, 18, pp. 779–798. doi: [10.1002/elsc.201800066](https://doi.org/10.1002/elsc.201800066)
- Goetzke, B.I., Spiller, A. (2014) ‘Health-improving lifestyles of organic and functional food consumers’ *British Food Journal*, 116 (3), pp. 510 – 526. doi: [10.1108/BFJ-03-2012-0073](https://doi.org/10.1108/BFJ-03-2012-0073)
- Han, K.H., Sekikawa, M., Shimada, K.I., Hashimoto, M., Hashimoto, N., Noda, T., Fukushima, M. (2006) ‘Anthocyanin-rich purple potato flake extract has antioxidant capacity and improves antioxidant potential in rats’ *British Journal of Nutrition*, 96(6), pp. 1125-1134. doi: [10.1017/BJN20061928](https://doi.org/10.1017/BJN20061928)
- Hao, G., Du, X., Zhao, F., Ji, H. (2010) ‘Fungal endophytes-induced abscisic acid is required for flavonoid accumulation in suspension cells of *Ginkgo biloba*’ *Biotechnology Letters*, 32(2), pp. 305-314. doi: [10.1007/s10529-009-0139-6](https://doi.org/10.1007/s10529-009-0139-6)
- Heim, M.A., Jakoby, M., Werber, M., Martin, C., Weisshaar, B., Bailey, P.C. (2003) ‘The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity’ *Molecular Biology and Evolution*, 20(5), pp. 735-747. doi: [10.1093/molbev/msg088](https://doi.org/10.1093/molbev/msg088)
- Hintz, T., Matthews, K.K., Di, R. (2015) ‘The use of plant antimicrobial compounds for food preservation’ *BioMed Research International*, 2015, 246264. doi: [10.1155/2015/246264](https://doi.org/10.1155/2015/246264)
- Hofvander, P., Andreasson, E., Andersson, M. (2021) ‘Potato trait development going fast-forward with genome editing’ *Trends in Genetics*, 1887. doi: [10.1016/j.tig.2021.10.004](https://doi.org/10.1016/j.tig.2021.10.004)
- Hosoda, K., Miyaji, M., Matsuyama, H., Haga, S., Ishizaki, H., Nonaka, K. (2012) ‘Effect of supplementation of purple pigment from anthocyanin-rich corn (*Zea mays* L.) on blood antioxidant activity and oxidation resistance in sheep’ *Livestock Science*, 145(1-3), pp. 266-270. doi: [10.1016/j.livsci.2011.12.001](https://doi.org/10.1016/j.livsci.2011.12.001)
- Jung, C.S., Griffiths, H.M., De Jong, D.M., Cheng, S., Bodis, M., Kim, T.S. (2009) ‘The potato developer (D) locus encodes an R2R3 MYB transcription factor that regulates expression of multiple anthocyanin structural genes in tuber skin’ *Theoretical and Applied Genetics*, 120, pp. 45–57. doi: [10.1007/s00122-009-1158-3](https://doi.org/10.1007/s00122-009-1158-3)
- Kallscheuer, N., Classen, T., Drepper, T., Marienhagen, J. (2019) ‘Production of plant metabolites with applications in the food industry using engineered microorganisms’ *Current Opinion in Biotechnology*, 56, pp. 7-17. doi: [j.copbio.2018.07.008](https://doi.org/10.1016/j.copbio.2018.07.008)
- Katsumoto, Y., Fukuchi-Mizutani, M., Fukui, Y., Brugliera, F., Holton, T.A., Karan, M., Tanaka, Y. (2007) ‘Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued

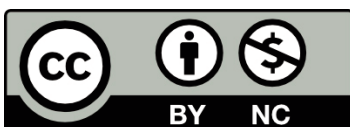
- flowers accumulating delphinidin' *Plant and Cell Physiology*, 48(11), pp. 1589-1600. doi: [10.1093/pcp/pcm131](https://doi.org/10.1093/pcp/pcm131)
- Khoo, H.E., Azlan, A., Tang, S.T., Lim, S.M. (2017) 'Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits' *Food & Nutrition Research*, 61, 1. doi: [10.1080/16546628.2017.1361779](https://doi.org/10.1080/16546628.2017.1361779)
- Koes, R., Verweij, W., Quattrocchio, F. (2005) 'Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. *Trends in Plant Science*, 10, pp. 236–242. doi: [10.1016/j.tplants.2005.03.002](https://doi.org/10.1016/j.tplants.2005.03.002)
- Lachman, J., Hamouz, K., Šulc, M., Orsák, M., Pivec, V., Hejtmánková, A. (2009) 'Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity' *Food Chemistry*, 114, pp. 836–843. doi: [10.1016/j.foodchem.2008.10.029](https://doi.org/10.1016/j.foodchem.2008.10.029)
- Landi, M., Tattini, M., Gould, K.S. (2015) 'Multiple functional roles of anthocyanins in plant-environment interactions' *Environmental and Experimental Botany*, 119, pp. 4-17. doi: [10.1016/j.envexpbot.2015.05.012](https://doi.org/10.1016/j.envexpbot.2015.05.012)
- Lei, C., Ma, Q., Tang, Q.Y., Ai, X.R., Zhou, Z., Yao, L., Dong, J.Z. (2014) 'Sodium selenite regulates phenolics accumulation and tuber development of purple potatoes' *Scientia Horticulturae*, 165, pp. 142-147. doi: [10.1016/j.scienta.2013.10.024](https://doi.org/10.1016/j.scienta.2013.10.024)
- Leisner, C.P., Hamilton, J.P., Crisovan, E., Manrique-Carpintero, N.C., Marand, A.P., Newton, L., Buell, C.R. (2018) 'Genome sequence of M6, a diploid inbred clone of the high-glycoalkaloid-producing tuber-bearing potato species *Solanum chacoense*, reveals residual heterozygosity' *The Plant Journal*, 94(3), pp. 562-570. doi: [10.1111/tbj.13857](https://doi.org/10.1111/tbj.13857)
- Leone, A., Costa, A., Tucci, M., Grillo, S. (1994) 'Adaptation versus shock response to polyethylene glycol-induced low water potential in cultured potato cells' *Physiologia Plantarum*, 92(1), pp. 21-30. doi: [10.1111/j.1399-3054.1994.tb06650.x](https://doi.org/10.1111/j.1399-3054.1994.tb06650.x)
- Levisson, M., Patinios, C., Hein, S. (2018) 'Engineering de novo anthocyanin production in *Saccharomyces cerevisiae*' *Microbial Cell Factories*, 17, 103. doi: [10.1186/s12934-018-0951-6](https://doi.org/10.1186/s12934-018-0951-6)
- Liu, Y., Lin-Wang, K., Deng, C., Warran, B., Wang, L., Yu, B. (2015) 'Comparative transcriptome analysis of white and purple potato to identify genes involved in anthocyanin biosynthesis' *PLoS ONE*, 10(6), e0129148. doi: [10.1371/journal.pone.0129148](https://doi.org/10.1371/journal.pone.0129148)
- Liu, Y., Tikunov, Y., Schouten, R.E., Marcelis, L.F.M., Visser, R.G.F., Bovy, A. (2018) 'Anthocyanin biosynthesis and degradation mechanisms in solanaceous vegetables: a review' *Frontiers in Chemistry*, 6, 52. doi: [10.3389/fchem.2018.00052](https://doi.org/10.3389/fchem.2018.00052)
- Ma, J., Xiang, H., Donnelly, D.J., Meng, F.R., Xu, H., Durnford, D., Li, X.Q. (2017) 'Genome editing in potato plants by agrobacterium-mediated transient expression of transcription activator-like effector nucleases' *Plant Biotechnology Reports*, 11(5), pp. 249-258. doi: [10.1007/s11816-017-0448-5](https://doi.org/10.1007/s11816-017-0448-5)
- Machida-Hirano, R. (2015) 'Diversity of potato genetic resources' *Breeding science*, 65(1), pp. 26-40. doi: [10.1270/jsbbs.65.26](https://doi.org/10.1270/jsbbs.65.26)
- Mano, H., Ogasawara, F., Sato, K., Higo, H., Minobe, Y. (2007) 'Isolation of a regulatory gene of anthocyanin biosynthesis in tuberous roots of purple-fleshed sweet potato' *Plant Physiology*, 143(3), pp. 1252-1268. doi: [10.1104/pp.106.094425](https://doi.org/10.1104/pp.106.094425)
- Marienhagen, J., Bott, M. (2013) 'Metabolic engineering of microorganisms for the synthesis of plant natural products' *Journal of Biotechnology*, 163, pp. 166–178. doi: [10.1016/j.jbiotec.2012.06.001](https://doi.org/10.1016/j.jbiotec.2012.06.001)
- Market Data Forecast (2021) '<https://www.marketdataforecast.com/market-reports/anthocyanins-market>
- Marone, D., Mastrangelo, A.M., Borrelli, G.M., Mores, A., Laidò, G., Russo, M.A., Ficco, D.B.M. (2022) 'Specialized metabolites: Physiological and biochemical role in stress resistance, strategies to improve their accumulation, and new applications in crop breeding and management' *Plant Physiology and Biochemistry*, 178, pp. 48-55. doi: [10.1016/j.plaphy.2021.12.037](https://doi.org/10.1016/j.plaphy.2021.12.037)
- Mekapogu, M., Vasamsetti, B.M.K., Kwon, O.K., Ahn, M.S., Lim, S.H., Jung, J.A. (2020)

- ‘Anthocyanins in floral colors: biosynthesis and regulation in Chrysanthemum flowers’ *International Journal of Molecular Sciences*, 21(18), 6537. doi: [10.3390/ijms21186537](https://doi.org/10.3390/ijms21186537)
- Mihovilovich, E., Sanetomo, R., Hosaka, K., Ordoñez, B., Aponte, M., Bonierbale, M. (2015) ‘Cytoplasmic diversity in potato breeding: case study from the International Potato Center’ *Molecular Breeding*, 35(6), pp. 1-10. doi: [10.1007/s11032-015-0326-1](https://doi.org/10.1007/s11032-015-0326-1)
- Montefiori, M., Brendolise, C., Dare, A.P., Lin-Wang, K., Davies, K.M., Hellens, R.P. (2015) ‘In the Solanaceae, a hierarchy of bHLHs confer distinct target specificity to the anthocyanin regulatory complex’ *Journal of Experimental Botany*, 66, pp. 1427–1436. doi: [10.1093/jxb/eru494](https://doi.org/10.1093/jxb/eru494)
- Murthy, H.N., Lee, E.J., Paek, K.Y. (2014) ‘Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation’ *Plant Cell, Tissue and Organ Culture*, 118(1), pp. 1-6. doi: [10.1007/s11240-014-0467-7](https://doi.org/10.1007/s11240-014-0467-7)
- Mustafa, N.R., de Winter, W., van Iren, F., Verpoorte, R. (2011) ‘Initiation, growth and cryopreservation of plant cell suspension cultures’ *Nature Protocols*, 6(6), pp. 715-42. doi: [10.1038/nprot.2010.144](https://doi.org/10.1038/nprot.2010.144)
- Oertel, A., Matros, A., Hartmann, A. (2017) ‘Metabolite profiling of red and blue potatoes revealed cultivar and tissue specific patterns for anthocyanins and other polyphenols’ *Planta*, 246, pp. 281–297. doi: [10.1007/s00425-017-2718-4](https://doi.org/10.1007/s00425-017-2718-4)
- Payyavula, R.S., Singh, R.K., Navarre, D.A. (2013) ‘Transcription factors, sucrose, and sucrose metabolic genes interact to regulate potato phenylpropanoid metabolism’ *Journal of Experimental Botany*, 64, pp. 5115–5131. doi: [10.1093/jxb/ert303](https://doi.org/10.1093/jxb/ert303)
- Pazderů, K., Hamouz, K., Lachman, J., Kasal P. (2015) ‘Yield potential and antioxidant activity of potatoes with coloured flesh’ *Plant, Soil and Environment*, 61(9), pp. 417-421. doi: [10.17221/416/2015-PSE](https://doi.org/10.17221/416/2015-PSE)
- Pekas, E., Shin, J., Headid, R., Son, W., Layec, G., Yadav, S., Park, S. (2021) ‘Combined anthocyanins and bromelain supplement improves endothelial function and skeletal muscle oxygenation status in adults: A double-blind placebo-controlled randomised crossover clinical trial’ *British Journal of Nutrition*, 125(2), pp. 161-171. doi: [10.1017/S0007114520002548](https://doi.org/10.1017/S0007114520002548)
- Peralta, I.E., Clausen, A.M., Zorrilla, C., Ames, M., Digilio, A., Rodriguez, F. (2021) ‘Wild and Cultivated Potato Species Diversity, Taxonomy, and Conservation’ In: Carputo, D., Aversano, R., Ercolano, M.R. (eds) ‘The Wild Solanums Genomes. *Compendium of Plant Genomes*. doi: [10.1007/978-3-030-30343-3\\_4](https://doi.org/10.1007/978-3-030-30343-3_4)
- Petric, T., Kiferle, C., Perata, P., Gonzali, S. (2018) ‘Optimizing shelf-life conditions for anthocyanin-rich tomatoes’ *PLoS One*, 13(10), e0205650. doi: [10.1371/journal.pone.0205650](https://doi.org/10.1371/journal.pone.0205650)
- Piao, C., Wu, J., Cui, M.L. (2021) ‘The combination of R2R3-MYB gene AmRoseal and hairy root culture is a useful tool for rapidly induction and production of anthocyanins in *Antirrhinum majus* L’ *AMB Express*, 11(1), pp. 1-9. doi: [10.1186/s13568-021-01286-6](https://doi.org/10.1186/s13568-021-01286-6)
- Pichersky, E., Lewinsohn, E. (2011) ‘Convergent evolution in plant specialized metabolism’ *Annual Review of Plant Biology*, 62(1), pp. 549-566. doi: [10.1146/annurev-arplant-042110-103814](https://doi.org/10.1146/annurev-arplant-042110-103814)
- Plaza, M., Pozzo, T., Liu, J.Y., Gulshan Ara, K.Z., Turner, C., Nordberg Karlsson, E. (2014) ‘Substituent effects on in vitro antioxidizing properties, stability, and solubility in flavonoids’ *Journal of Agricultural and Food Chemistry*, 62, pp. 3321–3333. doi: [10.1021/jf405570u](https://doi.org/10.1021/jf405570u)
- Prommachart, R., Belem, T.S., Uriyapongson, S., Rayas-Duarte, P., Uriyapongson, J., Ramanathan, R. (2020) ‘The effect of black rice water extract on surface color, lipid oxidation, microbial growth, and antioxidant activity of beef patties during chilled storage’ *Meat science*, 164, 108091. doi: [10.1016/j.meatsci.2020.108091](https://doi.org/10.1016/j.meatsci.2020.108091)
- Qu, J., Zhang, W., Yu, X. (2011) ‘A combination of elicitation and precursor feeding leads to increased anthocyanin synthesis in cell suspension cultures of *Vitis vinifera*’ *Plant Cell, Tissue and Organ Culture*, 107, pp. 261–269. doi: [10.1007/s11240-011-9977-8](https://doi.org/10.1007/s11240-011-9977-8)
- Ramakrishna, A., Ravishankar, G.A. (2011) ‘Influence of abiotic stress signals on secondary metabo-

- lites in plants' *Plant Signaling & Behavior*, 6, pp. 1720-1731. doi: [10.4161/psb.6.11.17613](https://doi.org/10.4161/psb.6.11.17613)
- Ramšak, Ž., Petek, M., Baebler, Š. (2021) 'RNA sequencing analyses for deciphering potato molecular responses' In: Dobnik, D., Gruden, K., Ramšak, Ž., Coll, A. (eds) *Solanum tuberosum. Methods in Molecular Biology*, 2354. doi: [10.1007/978-1-0716-1609-3\\_3](https://doi.org/10.1007/978-1-0716-1609-3_3)
- Rodriguez-Amaya, D.B. (2016) 'Natural food pigments and colorants' *Current Opinion in Food Science*, 7, pp. 20–26. doi: [10.1007/978-3-319-54528-8\\_12-1](https://doi.org/10.1007/978-3-319-54528-8_12-1)
- Sabbah, S., Tal, M. (1990) 'Development of callus and suspension cultures of potato resistant to NaCl and mannitol and their response to stress' *Plant Cell, Tissue and Organ Culture*, 21(2), pp. 119-128. doi: [10.1007/BF00033430](https://doi.org/10.1007/BF00033430)
- Sampaio, S.L., Lonchamp, J., Dias, M.I., Liddle, C., Petropoulos, S.A., Glamočlija, J., Barros, L. (2021) 'Anthocyanin-rich extracts from purple and red potatoes as natural colourants: Bioactive properties, application in a soft drink formulation and sensory analysis' *Food Chemistry*, 342, 128526. doi: [j.foodchem.2020.128526](https://doi.org/10.1016/j.foodchem.2020.128526)
- Sapko, O.A., Utarbaeva, A.S., Makulbek, S. (2011) 'Effect of fusaric acid on prooxidant and antioxidant properties of the potato cell suspension culture' *Russian Journal of Plant Physiology*, 58(5), pp. 828-835. doi: [10.1134/S1021443711050190](https://doi.org/10.1134/S1021443711050190)
- Shahidi, F., Ambigaipalan, P. (2015) 'Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects. A review' *Journal in Functional Foods*, 18, pp. 820–897. doi: [10.1016/j.jff.2015.06.018](https://doi.org/10.1016/j.jff.2015.06.018)
- Silva, S., Costa, E.M., Calhau, C., Morais, R.M., Pintado, M.E. (2017) 'Anthocyanin extraction from plant tissues: A review' *Critical Reviews in Food Science and Nutrition*, 57(14), pp. 3072-3083. doi: [10.1080/10408398.2015.1087963](https://doi.org/10.1080/10408398.2015.1087963)
- Simões, C., Albarello, N., Castro, T.C., Mansur, E. (2012) 'Production of anthocyanins by plant cell and tissue culture strategies' In: Orhan, I. (Ed.) *Biotechnological Production of Plant Secondary Metabolites*, Bentham Science Publishers, pp. 230-257.
- Speer, H., D'Cunha, N.M., Alexopoulos, N.I., McKune, A.J., Naumovski, N. (2020) 'Anthocyanins and human health - a focus on oxidative stress, inflammation and disease' *Antioxidants*, 9(5), 366. doi: [10.3390/antiox9050366](https://doi.org/10.3390/antiox9050366)
- Strygina, K.V., Kochetov, A.V., Khlestkina, E.K. (2019) 'Genetic control of anthocyanin pigmentation of potato tissues' *BMC genetics*, 20(1), pp. 35-43. doi: [10.1186/s12863-019-0728-x](https://doi.org/10.1186/s12863-019-0728-x)
- Stushnoff, C., Ducreux, L.J., Hancock, R.D., Hedley, P.E., Holm, D.G., McDougall, G.J. (2010) 'Flavonoid profiling and transcriptome analysis reveals new gene-metabolite correlations in tubers of *Solanum tuberosum* L.' *Journal of Experimental Botany*, 61, pp. 1225-1238. doi: [10.1093/jxb/erp394](https://doi.org/10.1093/jxb/erp394)
- Šulc, M., Kotíková, Z., Paznocht, L., Pivec, V., Hamouz, K., Lachman J. (2017) 'Changes in anthocyanidin levels during the maturation of color-fleshed potato (*Solanum tuberosum* L.) tubers' *Food Chemistry*, 237, pp. 981-988. doi: [10.1016/j.foodchem.2017.05.155](https://doi.org/10.1016/j.foodchem.2017.05.155)
- Swallah, M.S., Sun, H., Affoh, R., Fu, H., Yu, H. (2020) 'Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits' *International Journal of Food Science*, 2020, 9081686. doi: [10.1155/2020/9081686](https://doi.org/10.1155/2020/9081686)
- Tai, H.H., Goyer, C., Murphy, A.M. (2013) 'Potato MYB and bHLH transcription factors associated with anthocyanin intensity and common scab resistance' *Botany*, 91(10), pp. 722-730. doi: [10.1139/cjb-2012-0025](https://doi.org/10.1139/cjb-2012-0025)
- Tena, N., Martín, J., Asuero, A.G. (2020) 'State of the art of anthocyanins: Antioxidant activity, sources, bioavailability, and therapeutic effect in human health' *Antioxidants*, 9(5), 451. doi: [10.3390/antiox9050451](https://doi.org/10.3390/antiox9050451)
- Tengkun, N., Dongdong, W., Xiaohui, M., Yue, C., Qin, C. (2019) 'Analysis of key genes involved in potato anthocyanin biosynthesis based on genomics and transcriptomics data' *Frontiers in Plant Science*, 10, 603. doi: [10.3389/fpls.2019.00603](https://doi.org/10.3389/fpls.2019.00603)



- The Potato Genome Sequencing Consortium. (2011) ‘Genome sequence and analysis of the tuber crop potato’ *Nature*, 475, pp. 189–195. doi: [10.1038/nature10158](https://doi.org/10.1038/nature10158)
- Timmers, M.A., Grace, M.H., Yousef, G.G., Lila, M.A. (2017) ‘Inter-and intra-seasonal changes in anthocyanin accumulation and global metabolite profiling of six blueberry genotypes’ *Journal of Food Composition and Analysis*, 59, pp. 105-110. doi: [doi.org/10.1016/j.jfca.2017.02.019](https://doi.org/10.1016/j.jfca.2017.02.019)
- Tiwari, J.K., Devi, S., Ali, N., Luthra, S.K., Kumar, V., Bhardwaj, V., Chakrabarti, S.K. (2018) ‘Progress in somatic hybridization research in potato during the past 40 years’ *Plant Cell, Tissue and Organ Culture (PCTOC)*, 132(2), pp. 225-238. doi: [10.1007/s11240-017-1327-z](https://doi.org/10.1007/s11240-017-1327-z)
- Tonelli, M., Pellegrini, E., D’Angiolillo, F., Petersen, M., Nali, C., Pistelli, L., Lorenzini, G. (2015) ‘Ozone-elicited secondary metabolites in shoot cultures of *Melissa officinalis* L.’ *Plant Cell, Tissue and Organ Culture*, 120(2), pp. 617-629. doi: [10.1007/s11240-014-0628-8](https://doi.org/10.1007/s11240-014-0628-8)
- Van Swaaij, A.C., Talsma, K., Krigsheld, H., Jacobsen, E., Feestra, W.J. (1987) ‘Frost tolerance in cell cultures of potato’ *Physiologia Plantarum*, 69(4), pp. 602-608. doi: [10.1111/j.1399-3054.1987.tb01973.x](https://doi.org/10.1111/j.1399-3054.1987.tb01973.x)
- Vidana Gamage, G.C., Lim, Y.Y., Choo, W.S. (2021) ‘Sources and relative stabilities of acylated and nonacylated anthocyanins in beverage systems’ *Journal of Food Science and Technology*, pp. 1-15. doi: [10.1007/s13197-021-05054-z](https://doi.org/10.1007/s13197-021-05054-z)
- Zha, J., Wu, X., Koffas, M.A. (2020) ‘Making brilliant colors by microorganisms’ *Current Opinion in Biotechnology*, 61, pp. 135-141. doi: [10.1016/j.copbio.2019.12.020](https://doi.org/10.1016/j.copbio.2019.12.020)
- Zhang, H., Liu, R., Tsao, R. (2016) ‘Anthocyanin-rich phenolic extracts of purple root vegetables inhibit pro-inflammatory cytokines induced by H<sub>2</sub>O<sub>2</sub> and enhance antioxidant enzyme activities in Caco-2 cells’ *Journal of Functional Foods*, 22, pp. 363-375. doi: [10.1016/j.jff.2016.01.004](https://doi.org/10.1016/j.jff.2016.01.004)
- Zhang, Y., De Stefano, R., Robine, M., Butelli, E. (2015) ‘Different reactive oxygen species scavenging properties of flavonoids determine their abilities to extend the shelf life of tomato’ *Plant Physiology*, 169 (3), pp. 1568-1583. doi: [10.1104/pp.15.00346](https://doi.org/10.1104/pp.15.00346)
- Zhao, C.L., Yu, Y.Q., Chen, Z.J., Wen, G.S., Wie, F.G. (2017) ‘Stability-increasing effects of anthocyanin glycosyl acylation’ *Food Chemistry*, 214, pp. 119-128. doi: [10.1016/j.foodchem.2016.07.073](https://doi.org/10.1016/j.foodchem.2016.07.073)



© 2022 by the authors. Licensee Italian Society for Horticultural Science (Società di Ortoflorofruitticoltura Italiana; SOI), Sesto Fiorentino (Firenze), Italy. This work is an open access article distributed under a Creative Commons Attribution-NonCommercial (CC BY NC) 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>).