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Toward Human Health-Promoting Food Plants: Perspectives of Marker-Assisted Breeding of Anthocyanin-Rich Lettuce

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Abstract

Development and growing of plant cultivars with elevated content of health beneficial nutrients could improve the public human health. The aim of this study was to review the state of the art in use of molecular markers to produce anthocyanin-rich varieties of lettuce (*Lactuca sativa* L.). Link between anthocyanins production and their proved effect on the health was discussed. Although anthocyanin-dependent red colour of lettuce has been used in breeding for years the metabolic markers are not high-quality for noted purpose. Use of molecular markers can improve results of the breeding. Known and perspective DNA-based molecular markers were shown in this mini-review.

Keywords: lettuce, marker-assisted breeding, human health, molecular marker, anthocyanins, antioxidants, genetic profiling, single nucleotide polymorphisms, metabolic markers.

1. Introduction

Quality and composition of food is extremely important for human health. At least 22 % of all death in adult population all over the world are resulted from action of dietary risk factors (GBD 2017 Diet Collaborators, 2019). Sufficient daily intake of vegetables (optimal level is 290–430 g per day) have benefits for health. On the other hand, increased nutrients content in vegetables can improve health conditions even in the case of low vegetables consumption that recognized as one of five strongest dietary risk factors. Nutrient composition of vegetables is very variable and determined by genetic and environmental factors both. Therefore, elevated production of human health beneficial nutrients is important goal in breeding of cultivated plants (Hansson et al., 2018).

Anthocyanins, a subgroup of flavonoids, are plant secondary metabolites with proven benefits for human health (Khoo et al., 2017; Yang et al., 2017; Rees et al., 2018). However, concentration of anthocyanins in plant tissues can vary dramatically and is controlled by multiple exogenous stimuli like light intensity, salt concentration, humidity, temperature and so on (Liu et al., 2018). Specified variability is especially meaningful in leafy vegetables that require development of new cultivars with sustained high production of anthocyanins.

The aim of this study was to review the state of the art in use of molecular markers to produce anthocyanin-rich varieties of lettuce (*Lactuca sativa* L.).

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2. Anthocyanins

2.1. Structure and distribution in nature

Anthocyanins are particular members of the flavonoid family among plant phenols. Anthocyanins are glycosylated polyphenolic compounds found in a lot of plants and giving them various colours – from orange and red to blue and purple. Plants contains little amounts of aglyconic forms of anthocyanins called antocyanidins as anthocyanidins are precursors in biosynthesis of anthocyanins. Both anthocyanidins and antocyanins are positive charged and coloured under physiological conditions but only glycosylated compounds can be accumulated into vacuoles (Castañeda-Ovando et al., 2009). More than 700 anthocyanins have been found in plants to the date (Smeriglio et al., 2016; Santos-Buelga, González-Paramás, 2019). Glycosides of cyanidin, pelargonin, delphinidin, peonidin, petunidin and malvidin are most common anthocyanins in plants (Khoo et al., 2017).

Anthocyanins play multiple physiological roles in plants. Firstly, they colourize flowers to attract pollinators and edible fruits to improve the chances of seed dissemination by animals eating fruits, therefore anthocyanins are important for plant reproduction (Pervaiz et al., 2017). Secondary, anthocyanins are involved in physiological response to various biotic and abiotic stresses. Influence of many dangerous factors including drought, heavy metals, pathogenic viruses and microbes invasion can be diminished by anthocyanins. Moreover, anthocyanidins have antioxidative properties and effectively scavenge free radicals and reactive oxygen species produced in particular during UV and light-induced photodamage (Gould, 2004).

Contents of anthocyanins in edible plants vary widely – from less than 10 µg/g to about 14 mg/g (Santos-Buelga, González-Paramás, 2019). Common food sources of anthocyanidins are brightly-coloured fruits and berries containing cyanidin derivatives like apple, plum, cherry, blackberry, raspberry, strawberry (Andersen, Jordheim, 2013). Blackberry, blueberry, black currant and chokeberry have the highest concentration of anthocyanins among edible berries and (Santos-Buelga, González-Paramás, 2019) and black carrot, red cabbage, black soybeans, purple batat and purple potato among vegetables (Khoo et al., 2017).

2.2. Effect on human health

Some human health beneficial effects of anthocyanins were proved and a significantly larger number of biological activities were tested in experimental studies and clinical trials (Smeriglio et al., 2016; Khoo et al., 2017). Li et al. summarized results of clinical trials and noted anthocyanins from fruits and vegetables can decrease risk of development or progression of some cancers including tumours of breast, prostate, liver, colon, lungs, cervix as well as metastatic melanoma (Li et al., 2017). Antiinflammatory properties, neuroprotective action of anthocyanins and their benefits for cognition and memory were discussed too (Mulabagal et al., 2010; Li et al., 2017).

Some clinical trials showed attenuating effects of anthocyanins on human cardiovascular risk factors including elevated blood pressure and dysfunction of vessel endothelium but other studies exhibited controversial results (Rees et al., 2018). Possible mechanisms of action include decline of low density lipoproteins oxidation, elevation of blood antioxidant capacity, and attenuation of dyslipidemia (Reis et al., 2016). There are evidences of antiobesity and antidiabetic effects of anthocyanins from experimental and clinical studies (Azzini et al., 2017; Lee et al., 2017; Yang et al., 2017).

Therefore, anthocyanins have multiple positive effects on human health and long-term consumption of vegetable and fruits containing more anthocyanins could prevent some cases of disease development without use of pharmaceuticals.

2.3. Anthocyanins in lettuce

Lettuce (*Lactuca sativa* L.) is one of the widely used leaf vegetables and is a member of the Asteraceae family (Anilakumar et al., 2017). Lettuce has short vegetation period and can be easy grown in field or soil-based greenhouse as well as by use of hydroponic, aeroponic or vermiponic systems (Barbosa et al., 2015; Bartzas et al., 2015). There are green and red varieties of lettuce and the last ones can accumulate anthocyanins in leaves and stem. Anthocyanins content in lettuce can vary very widely: from {negligible or trace amounts} 1.9 µg/g fresh weight in green varieties (Mampholo et al., 2016) to 874.4 µg/g dry weight in red or purple ones (Gazula et al., 2007). Different species of plants contain limited number anthocyanins although chemical diversity of

them is great (Chaves-Silva et al., 2018). Glucosides of cyanidin and peonidin were detected as major anthocyanins in *Lactuca sativa*.

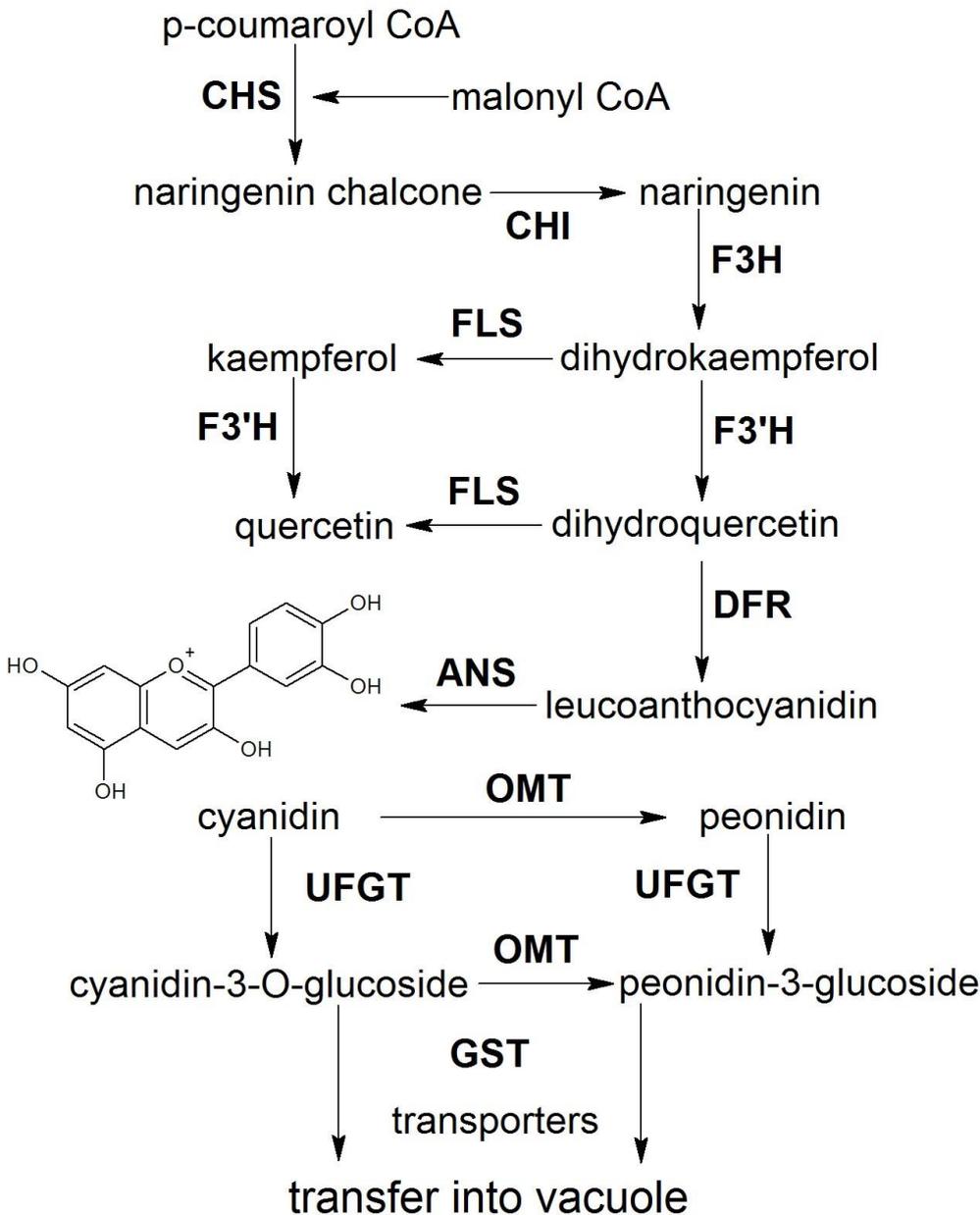


Fig. 1. Metabolic pathway of flavonoids and anthocyanins biosynthesis in lettuce. See abbreviation in the text

Biosynthesis of anthocyanins in lettuce (Figure 1) needs sequential formation of some flavonoids as precursors (Winkel-Shirley, 2001; Pervaiz et al., 2017). Consequently, this biochemical pathway requires the participation of following enzymes noted on Figure 1: chalcone synthase (CHS, EC: 2.3.1.74), chalcone isomerase (CHI, EC: 5.5.1.6), naringenin 3-dioxygenase or flavonone 3-hydroxylase (F3H, EC: 1.14.11.9), flavonol synthase (FLS, EC: 1.14.20.6), flavonoid 3'-monooxygenase or flavonoid 3'-hydroxylase (F3'H, EC: 1.14.14.82), dihydroflavonol 4-reductase (DFR, EC: 1.1.1.219), anthocyanidin synthase (ANS, EC: 1.14.20.4, also known as leucocyanidin oxygenase or leucoanthocyanidin dioxygenase - LDOX), anthocyanidin 3-O-glucosyltransferase or flavonoid 3-O-glycosyltransferase (3GT or UFGT, EC: 2.4.1.115) and glutathione S-transferase (GST, EC 2.5.1.18). Enzymes from CHS to F3'H are components of flavonoid metabolism pathway and - excluding FLS - involved in synthesis of dihydroquercetin, a immediate precursor of lettuce anthocyanidins. DHF and ANS produces cyanidin, a principal anthocyanidin of lettuce (Winkel-

Shirley, 2001; Pervaiz et al., 2017). Glycosylation of anthocyanidins to anthocyanins is necessary for following their translocation into vacuoles with the assistance of GST (or GST-like proteins) and multi-drug resistance-like proteins from ABC transporter family (Tanaka et al., 2008). Cyanidin and cyanidin 3-glucoside can be transformed in peonidin and peonidin 3-glucoside respectively by unidentified O-methyltransferase(s) (OMT) possibly like anthocyanidin OMT from other plants (Provenzano et al., 2014).

Rate of the anthocyanins formation is highly variable between various cultivars of lettuce, between different plants of the same cultivar and even in individual plant during its development since production of anthocyanins plays key role in plant adaptation especially for adaptation to local ecosystems (Mouradov, Spangenberg, 2014). Therefore, anthocyanins biosynthesis rate is closely controlled by multiple environmental factors including intensity and spectral properties of light, temperature, humidity, presence of various substances in soil and air, microbial or viral infections. These factors can influence both total concentration and composition of anthocyanins in lettuce (Becker et al., 2014; Brücková et al., 2016; Kitazaki et al., 2018).

3. Marker-assisted breeding of lettuce for anthocyanin enrichment

Once anthocyanins give lettuce red colouration the colour of leaves and stem have been using in lettuce breeding for years. Typically, lettuce colouration can be estimated visually (Sochor et al., 2019). It is very subjective test. There have been attempts to improve measurement of anthocyanins content by use of biochemical assays (Şakar et al., 2008; Volden et al., 2009), reflective photometry (Gazula et al., 2007; Volden et al., 2009) or digital image analysis (Yang et al., 2016). Whatever the case, metabolic profiling/phenotyping in case of anthocyanins can give strong bias due to production of the anthocyanins is markedly dependent upon a lot of environmental factors. Differences in anthocyanins content in plant of the same cultivar can be up 1.5-fold in the same year and larger than 2-fold between different years (Gazula et al., 2007). Use of expressed mRNA or proteins as markers can be inappropriate for the same reasons. Use of DNA-based molecular markers can improve results of the marker-assisted breeding (Collard, Mackill, 2008; Nadeem et al., 2018).

Unfortunately, there are just a few studies for screening of DNA-based molecular markers associated with anthocyanins production in lettuce. Zhang et al. (Zhang et al., 2017) used transcriptome analysis of 163 cultivars to find candidate genes associated with flavonoid biosynthesis regulation. In theory, any of genes involved in biosynthesis of flavonoids including anthocyanins (see section 1.3) could be related with forming with anthocyanin-dependent red colouring in lettuce plants. However, Zhang et al. found only genes for ANS and GST had higher level of expression in red lettuce comparing to green lettuce. Additionally, expression levels of genes MYB113 (encoding one of MYB family transcription factors), bHLH42 (encoding transcription factor TT8) and LG1_162414 (encoding RING/U-box superfamily protein) were related with anthocyanins positively. Their products are regulatory proteins probably involved in plant adaptation processes. Only gene encoding cinnamyl-alcohol dehydrogenase (CAD) correlated to red colouration of lettuce leaves negatively. Nevertheless, statistically identified anthocyanin synthesis up-regulation single nucleotide polymorphisms (SNPs) were partially related to candidate genes above. In fact, only SNP in position 125530709 on chromosome LG1 was inside LG1_162414 gene the expression of which correlated with anthocyanin accumulation. Other 8 SNPs located on chromosomes LG3, LG4 and LG5 were not associated with any of the candidate genes (Zhang et al., 2017).

Genome-wide analysis of 298 lines of lettuce allowed to find 4 SNPs associated with anthocyanins levels. Particularly noteworthy was the fact that all found SNPs were related to content of anthocyanins in leaves but only 2 of them were additionally associated with anthocyanins concentration in stem (Kwon et al., 2013). Five cultivars of cultivated lettuce and 60 recombinant inbred line generated from a cross between cultivated lettuce (*L. sativa*) and wild *L. serriola* were used for search of quantitative trait loci (QTLs) related to antioxidant status. Three QTLs associated with anthocyanins production and red colouring were found on chromosome LG3. Two QTLs were observed inside genes of MYB family transcription factor: production of anthocyanin pigment 2 (PAP2) protein also known as MYB90 and MYB114. One more QTL was identified inside gene for F3H, enzyme involved in flavonoid biosynthesis. All three QTLs can be used as DNA-based molecular markers in cultivated *L. sativa* and wild *L. serriola* both (Damerum

et al., 2015). MYB transcription factor are well known as regulator of the dihydroflavonols to anthocyanidins conversion (Allan et al., 2008).

In general, use of wild species of genus *Lactuca* can give additional benefits in anthocyanin-rich lettuce breeding for two main reasons: (1) various *Lactuca* species can be hybridized with cultivated lettuce relative easy, and (2) expression levels of some anthocyanin-related markers in wild *Lactuca* spp. are higher than in *L. sativa* (Damerum et al., 2015).

4. Conclusion

1. High content of the anthocyanins in edible plants can attenuate some disease and improve the public human health.
2. Use of metabolic, protein-based and mRNA-based markers in breeding of anthocyanin-rich lettuce cultivars can lead to limited success due to large variations in anthocyanins content under the effect of multiple epigenetic factors.
3. Use of DNA-based molecular markers is more appropriate.
4. Three QTLs and 13 SNPs associated with anthocyanin production in *Lactuca sativa* have been identified.
5. Hybridization of cultivated lettuce with wild *Lactuca* spp. can give additional benefits for anthocyanin-rich cultivars molecular selection.

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