Hypotheses

Can Anthocyanins be Part of the Metal Homeostasis Network in Plant?

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Abstract: Anthocyanins are a class of flavonoids with a high level of diversification and likely the most studied pathway of secondary metabolism in *Plantae*. Anthocyanins have raised a growing interest due to the huge variability of their chemical structures and the more new anthocyanins are isolated from plants, the more questions on their evolutionary and ecological meaning they raise. Antioxidant, photoprotection against high light and UV, defence against herbivores and pathogens, attraction of pollinator are only some proposed biological functions for those versatile compounds. Anthocyanins have also been found complexed with metal ions either in flower pigments (commelin and protocyanin) or in leaves and stems. Due to the potentiality of anthocyanins to chelate to metals, their involvement in the attenuation of metal toxicity has been recently proposed. Conversely, the ability of plants to remobilize metal ions from stored metal-anthocyanin complexes when plants experience a period of transient metal shortage has never been investigated before. The aim of this paper is to support the hypothesis that the anthocyanin-metal interactions might represent a further ecological role for these pigments and also that anthocyanins can be part of the complex network of metal homeostasis in plant.

Keywords: Anthocyanins, Flavonoids, Metallo-Anthocyanins, Metal Shortage, Land Plants, UV Waveband

Introduction

Anthocyanins are the plant-derived flavonoids which are responsible for the marvellous colours of plant organs, ranging from pale pink to red to purple and deep blue. They are present in a wide range of plant tissues, principally flowers and fruit, but also storage organs, roots, tubers and stems. Anthocyanins biosynthesis is likely the most studied secondary metabolite pathway in plants and the genes have been isolated for almost every biosynthetic step (Andersen and Jordhein, 2006).

The physiological role(s) of anthocyanins in plants have intrigued scientists for well over a century. To date, despite a lot of knowledge has been gained on matter, the presence of anthocyanins in almost all organ cells and the wide distribution of these pigments in the *Plantae* kingdom remains far from obvious (Gould *et al*., 2009). Despite it is undeniable that the selective pressure has driven evolution to anthocyanins in such disparate vegetative organs and that angiosperms evolution is parallel by the evolution of anthocyanins, the selective factors that led to the production of these pigments remain partially a mystery. As for other physiological mechanisms, the comprehension of the role of anthocyanins during the evolution would require the exploration and the contextualization of their functions to early stages of plant's evolution. However, this approach is complicated by (i) the reductionist approach intrinsic in the scientific method (even the best experimental model cannot take into consideration all the variables that might have influenced the process); (ii) the difficult of understanding the evolutionary meaning of an adaptation mechanism in early stage of evolution as many interactions among factors which occurred in the past might have been lost, changed, or still undiscovered.

Considering the high number of publications on the evolutionary meaning(s) of anthocyanins in plants, this paper does not presume to explain such complicated mechanisms, but it has been written with the attempt to provide a new realistic point of view on the role of metal-

anthocyanin complexes in plants, with the aim to attract scientific interest and stimulate researches on matter.

The Key Role of Phenols in Terrestrial Plants Evolution

It is generally accepted that the development of phenylpropanoid metabolism played a key role in the evolution of terrestrial plants and it allowed them to survive under high UV radiations (Rozema *et al*., 2002). Before the mid-Palaeozoic era, between about 480 and 360 million years ago, when first land plants appeared and started to evolve, life was restricted to aquatic ecosystems where UV-B filtering was exerted by substances dissolved or suspended in the water column (Kenrick and Crane, 1997). Due to the lack of a significant screening of the Earth's surface from the effects of UV radiation, first land plants had to counteract the negative effects of UV, especially UV-B. Oxygen in large amounts first had to be generated by the photosynthesis of land plants before the UV-screening ozone layer was created (Rozema *et al*., 2002).

Phenolic acids are the first by-products of phenylpropanoid metabolism and probably they represented the first barrier against UV radiation in first terrestrial plants arose from the aquatic Chlorophyceae group (Kenrick and Crane, 1997). Indeed, phenolic acids were already synthetized by aquatic organisms despite the UV radiation reaching them under the water surface was drastically reduced compared to that present on Earth's surface (Stafford, 1991). The latter author reported that the UV filtering role proposed also for flavonoids is attractive, but to act in that way flavonoids would require accumulating at relative high concentration. Presumably, the first flavonoids synthetizing enzymes were not so efficient to provide that concentration in the first species of land plants (Stafford, 1991). However, one has to consider that the acclimation from aquatic ecosystems to land occurred progressively and it is at least presumable that during that period, in addition to phenolic acids, other molecules (such as flavonoids), even though less efficient in terms of UV molar absorption, enhanced basic protection from UV. Thus, first flavonoids might have conferred a further protection to plant which firstly evolved genes for flavonoids biosynthesis. That UV radiation has many times been demonstrated as responsible for the enhancement of Phenylalanine Ammonia Lyase (PAL), chalcone synthase and other key enzymes of the phenylpropanoid pathway is likely a demonstration of the correlation between phenols and UV. Of note, PAL catalyzes the formation of *trans*-cinnamic acid that has a molar absorption coefficient higher than phenylalanine from which cinnamic acid originates (Rozema *et al*., 2002).
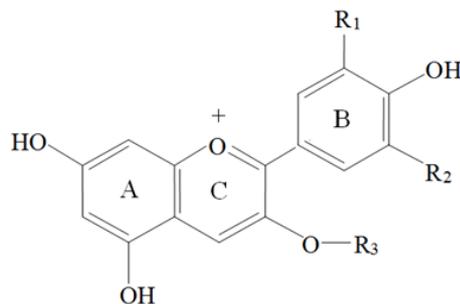
The evolutionary advantages of phenols in plants did not end with the UV-filtering properties. The complexity of phenols has indeed progressively increased with the evolutionary advancement from bryophytes, pteridophytes, gymnosperms to angiosperms, respectively; the polymerization of phenols diversified new compounds during evolution (flavonoids, lignin, tannins) (Boudet, 2012). Those molecules started to confer advantages for plants in such different ways: Allelopathy, hormones, defense against pathogens and herbivores and structural rigidity (Strack, 1997). To date, lot remains still unknown about phenols in plants, but among all the flavonoids the ecological meaning of anthocyanins is likely the most debated and intriguing.

Why are Anthocyanins Widely Diffused in Plantae and why are they Diversified in Hundreds of Compounds?

A unified explanation for the presence of anthocyanins in plants is impossible as for each species the presence of anthocyanins cannot be described isolating those pigments neither from the plant metabolic framework nor from the ecological niche of the species under investigation. Thus, despite identical histological localization and chemical composition of anthocyanins in plant tissue, any two species might receive different benefits from anthocyanins in such a different way and degree. Also, in two species with a different anthocyanin profile those compounds might exert similar effects. Starting from those evidences, over the last decades many researches have been addressed to the exploration of the behaviour of anthocyanic plants under different environmental conditions.

These flavonoids are glycosides of flavylium or 2-phenylbenzopyrylium salts and are commonly based on six anthocyanidins: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. The sugar moiety can vary, but is commonly constituted by glucose, rhamnose, galactose or arabinose. In addition, it may be acylated with a phenolic or aliphatic acid. These compounds differ in the methoxyl and hydroxyl substitution pattern of ring B (Fig. 1). While there are six common anthocyanidins, there are more than 600 anthocyanins isolated from plants (Guidi *et al*., 2015), 277 of which have been identified after 1992 (Andersen and Jordheim, 2006). In view of the chemical structure of anthocyanins and their histological localization, different protective functions have been hypothesized even though, concerning plant protection against abiotic stress, photoprotection from high light and UV-B (Agati *et al*., 2007; Tattini *et al*., 2014) and ROS scavenging ability are the most widely postulated as stress reliever (Gould *et al*., 2002; Kytridis and Manetas, 2006). On the other hand, defence against herbivores and attraction of pollinators (or preys in carnivorous plants) are the most proposed functions for anthocyanins in terms of relationships with biotic factors (Gould *et al*., 2009; Hughes and Lev-Yadun, 2015; Menzies *et al*., 2015).

Antocyanin	R_1	R_2	λ max (nm)	
			R_3=H	R_3=glucoside
Pelargonidin	H	H	520	516
Cyanidin	OH	H	535	530
Peonidin	OCH_3	H	532	528
Delphinidin	OH	OH	546	541
Petunidin	OH	OCH_3	543	540
Malvidin	OCH_3	OCH_3	542	538

Fig. 1. The most abundant anthocyanidins in nature, the substituted of flavylium cation, the typical colour and the maximal absorbance (λ max; nm) in acidified methanol (0.01% HCl)

Despite the impossibility to find an universal role for these flavonoids, what in many cases anthocyanic species have in common is the up-regulation of the biosynthesis of those compounds as a cross response to different environmental constrains such as high light, UV-B waveband, drought, high temperatures, ozone, bacterial and fungal infections, wounding, herbivores, herbicides and many other pollutants (i.e., heavy metals) (for review see Landi *et al*., 2015). Such instance, pigmented plants utilized for phytoremediation have shown to increment the level of anthocyanins with dependence to the concentration of some metal ions (Cd, Co, Cu, Mg, Ni, Zn, Pb, Cr) in soil or nutrient solution (Nanda-Kumar *et al*., 1995; Chalker-Scott, 1999). Sometimes, it has been observed that also the metal deficiency increases the biosynthesis of anthocyanins in plant tissue (Hermans *et al*., 2006). In addition to their stimulation by metal imbalance, anthocyanins biosynthesis is also up-regulated in many plant species in response to macronutrient shortage (Marschner, 1995), particularly N and Pi deficiencies (Stewart *et al*., 2001; Nikiforova *et al*., 2004; Peng *et al*., 2008; Kovinich *et al*., 2014). Under condition of mineral imbalance, it has been demonstrated many times that anthocyanins attenuate the magnitude of the induced stress on cellular processes (in particular photosynthesis) acting as photoprotectors and/or free radicals scavenger (for reviews see Steyn *et al*., 2002; Gould, 2010; Landi *et al*., 2015). Conversely, other roles by which anthocyanins might ameliorate the effect of nutrient imbalance on plant metabolism have been less studied. Among them, the ability of anthocyanins to chelate metals or metalloids when those elements exceed the plant's requirement has been recently proposed (Landi *et al*., 2014; 2015; Pardossi *et al*., 2015) and this property will be discussed below. In addition, the hypothesis that anthocyanins can be part of an orchestrated network of metal homeostasis is also explored herein.

Anthocyanins and Metal Complexes: From Willstätter to Today

First evidences of the metal-chelating ability of anthocyanins date back to 1919 when Shibata and co-workers found when a flavonol was reduced with Mg^{2+}, a blue-green solution was obtained (Shibata *et al*., 1919). A chemical analysis of the colouring matter indicated that the blue colour was due to Mg^{2+}-anthocyanin complexes and they postulated the metal-complex theory for blue colour development of *Centaurea cyanus* flowers. That hypothesis disproved the previous one of Willstätter and Everest (1913), which proposed the pH-dependent theory for blue flower colour development of *C. cyanus*. Indeed, in 1913 Willstätter and Everest isolated a blue pigment that they named "cyanin" from that species and in 1915 they found the same pigment in rose (Willstätter and Nolan, 1915) and attributed the colour variation to differences in pH. Red is typical of acidic solution and blue of alkaline ones. Shibata *et al*. (1919) disproved that hypothesis because petal flower are just slightly acid and the authors proposed the theory which involved complex of anthocyanins with Mg^{2+} ions. However, the theory of Shibata and co-workers was ignored by European chemistry in the early part of the 20th century (Yoshida *et al*., 2009).
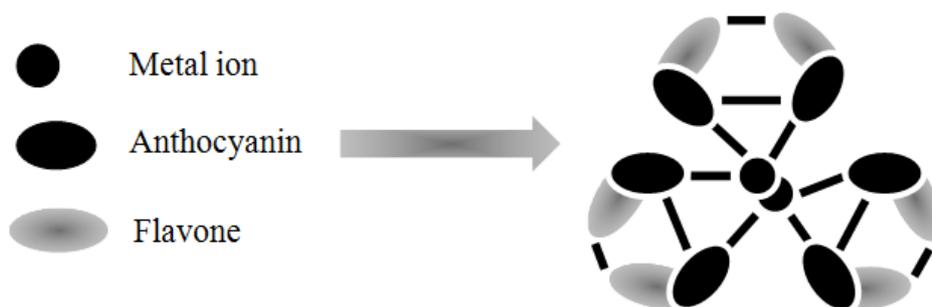
Fig. 2. Tipycal structure of a Metallo-Anthocyanin (MA). 2 metal ions, 6 anthocyanins and 6 flavones are self-assembly in a single stable compound

Only several years later the theory of the Shibata brothers became realistic, when Hayashi (1957) and Hayashi *et al.* (1958) isolated a blue anthocyanin pigment from *Commelina communis* (they named the pigment "commelinin") and they demonstrated that commelinin is a MA composed by an anthocyanin moiety, flavones and ions Mg^{2+}. However, only at the end of the 20th century Kondo *et al.* (1992) determined the full structure of commelin by X-ray crystallography. The term MA refers to a self-assembled, supra-molecular metal complex pigment; this complex is composed of stoichiometric amounts of anthocyanins, flavones and metal ions (6:6:2, respectively) and the presence of metal ions is essential for the complex formation (Takeda and Hayashi, 1977) (Fig. 2).

In addition to commelinin and protocyanin, several MAs that involve other metal ions, such as Al^{3+} (blue hydrangea petals; Kondo *et al.*, 1994) or Fe^{3+} (blue petals of *Tulipa gesneriana*; Shoji *et al.*, 2007), have been isolated in flowers (for a review see Yoshida *et al.*, 2009). Commelinin-like supramolecules, in which one or more components are replaced, have also been artificially reconstituted. Such instance, Mg^{2+} was replaced by Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} (Harborne and Williams, 2000; Nigel and Grayer, 2008), suggesting that under circumstances of deficiency of a particular microelement, plant can replace it with another ion with similar chemical features. However, despite the ions replacement can occur, depending on the type of ions the UV-vis optical properties may have a wide range of variation (Yoshida *et al.*, 2009).

Anthocyanin-metal complexes have been found not only in MAs but also in simpler compounds. The ability of anthocyanins to chelate to metals has been reported for W (Hale *et al.*, 2002), Al (Elhabiri *et al.*, 1997), Cu (Somaatmadja *et al.*, 2006), Ga (Elhabiri *et al.*, 1997; Buchweitz *et al.*, 2012), Fe (Buchweitz *et al.*, 2012), Mo (Hale *et al.*, 2001). The latter research also demonstrated that the accumulation of Mo in *Brassica rapa* is related to the genotype's anthocyanin-production ability and the anthocyanin-less genotype

exhibited the lowest accumulation of Mo within tissues. The ability of anthocyanins to chelate to metal ions is due to the 3'-4' ortho-dihydroxyl group on the B ring of the anthocyanin (Fig. 1) where the bind occurs. Recently, the same ability of foliar anthocyanins has also been postulated for boron that, differently to the elements previously mentioned, is a metalloid (Landi *et al.*, 2014; 2015; Pardossi *et al.*, 2015).

Sequestration of toxic metals in peripheral cell layers or their exclusion from cytosolic space (i.e., due to apoplast accumulation and vacuolar storage) are mechanisms commonly adopted by plant to concentrate metals in plant tissues where they are less harmful (Hale *et al.*, 2002). In addition, when anthocyanins bind to metal ions, the complexes which originate may have a strongly increment in UV absorption with respect to the anthocyanin moiety alone (Hale *et al.*, 2002; Yoshida *et al.*, 2009). The ability of metal-anthocyanins to absorb UV wavebands can represent a further line of defense against the excess of harmful UV, especially when chloroplasts resulted impaired by the effects of metal toxicity.

Anthocyanins as Part of Plant Metal Homeostasis Network: Is this a new Realistic Possibility for their Ecological Meaning?

First plants on land colonized environments with optimal conditions for their life in terms of light exposure, temperature, water and nutrient availability. Over millennia, plants have progressively evolved, acquiring new features (either morphological or biochemical/physiological) allowing them to explore and colonize new ecological niches, including also environments subjected to harsher conditions vacated by more primitive species.

It is generally accepted that the evolution is driven by selective pressure mechanisms and is commonly oriented toward models of living organisms with (i) higher levels of complexity and (ii) more efficient energy utilization and resources allocation than that of the predecessor species. From that point of view the development of "the new biosynthetic pathway of anthocyanins" that

progressively has developed in angiosperms, reflects exactly this concept. Firstly, because anthocyanins originated from hydroxycinnamic acids and, as all the other flavonoids, they have evolved in more complexes chemical structures. Secondly, in addition to the protection against UV, especially due to acylation with phenolic acids (i.e., coumaric acid; Tattini *et al*., 2014), they serve as visible light filter as well as powerful antioxidants (Gould *et al*., 2009; Landi *et al*., 2015). Thus, anthocyanins exhibit properties of other different classes of molecules, but enclosed in a single one confirming the second criterion of the evolution: the efficiency of energy utilization. From an evolutionary point of view the metal-chelation ability represent a further step towards incremented metabolic efficiency and versatility of anthocyanins and MAs are likely one of the best example. Flavone and anthocyanin moieties present in MAs might insure the protection of flower against excess of visible wavebands, the phenolic acids increase the protection against UV and the metal ions represent the means to complex and stabilize flavonoid and anthocyanin moieties together in a single and high-stable compound. But if a metal-sequestration capacity can be ascribed to anthocyanins in plants experiencing metal excess, could the role of anthocyanins be more sophisticated than a mere sequestration when plants face fluctuation of metal availability? This condition can occur, for example, under prolonged hot and dry periods in which the metal solubility is limited by reduced soil water availability or due to temporary metal immobilization in soil caused by transient changes of soil pH.

Concentration and availability of nutrients, especially trace metals, might have represented a relevant limiting factor for plant survivor due to the high number of biological processes in which metals are directly (or indirectly) involved. Metals are necessary for plant's metabolic reactions (i.e., enzymes activities, electron transport chains, etc.) (Marschner, 1995), and also for the development of flower's pigmentation in some plant species (Yoshida *et al*., 2009). Such instance, almost half of all enzymes must associate with a particular metal to function (Waldron *et al*., 2009). However, these same metals can be toxic at supra-optimal concentrations. In order to maintain the concentration of metal in plant tissues within a physiological range, plant have evolved (and diversified) a complex network of mechanisms to maintain this homeostasis (Clemens, 2006). The main detoxification pathway for metals in plants involves the conjugation to glutathione by glutathione S-transferase as well as the biosynthesis of glutathione-derived peptides (namely phytochelatins) which can facilitate either the vacuolar accumulation of metals (Clemens, 2006; Yadav, 2010) or also their long distance transport between shoot and roots (Brunetti *et al*., 2011). Interestingly, glutathione S-transferases has been recognized as responsible either for accumulation of

anthocyanins or metal ions into the vacuole (Alfenito *et al*., 1998). Thus, Landi *et al*. (2015) proposed that anthocyanins conjugated with glutathione might transiently bind to metal (or metalloid) ions in the cytosol, forming glutathionyl-anthocyanin-metal complexes. The formation of this adduct would be energetically efficient in terms of the glutathione balance of a cell given that, instead of requiring one molecule of glutathione to complex with a molecule of anthocyanin and another glutathione molecule (or even two) to sequestrate a metal ion, the glutathionyl-anthocyanin-metal mechanism would require only a single molecule of glutathione to transport an anthocyanin-metal (or anthocyanin-metalloid) (for more details see Landi *et al*., 2015). This would allow maintaining higher level of free glutathione that could be useful utilized to store other metal ion, thus reducing the toxic effect of high metal concentrations in the cytosolic compartment.

In some cases, after a period of luxury availability of nutrients (including metals), plants can experience long periods in which the availability of some metals can be strongly reduced due to environmental conditions which limit for example their solubilization and/or root uptake. Normally, plant can remobilize metal ions from vacuole due to specialized transporters, such as those belonging to the NRAMP family (Briat *et al*., 2007; Kramer *et al*., 2007) as observed for example when plant experiences metal starvation (Briat *et al*., 2007). A similar remobilization can occur also from old to younger leaves during senescence. Thus, plant's metal sensing and homeostasis involve a complex and orchestrated network of metabolic pathways. However, metal remobilization has been found to occur for free unbind metal ions (Briat *et al*., 2007; Kramer *et al*., 2007); different can be the situation when plants accumulated high amount of metals inside the vacuoles as anthocyanin-metal complexes. In this case, metal recycling would require previously the disruption of the metal-anthocyanin bind (such instance with the enzymatic activity of some hydrolases) before the ion could be export from vacuole by dedicated transporters. To the best of my knowledge, no reports have explored the possibility that metal ions can be released by the anthocyanin-metal complexes and to date the role of anthocyanins under fluctuation of plant's metal availability has never been investigated. That under metal shortage metal-anthocyanin complexes could be fragmented into their moieties releasing the metal ion and making it available for plant metabolic requirements would warrant future investigation for different reasons. Firstly, because in case the release of metal ion would occur, it would suggest a more sophisticated role for anthocyanins in metal homeostasis. Indeed, the signal which would activates the hydrolyses of metallic ions would implicate the presence of a specific metal-sensing at whole cell level.

This role for anthocyanins would further increase the versatility of these compounds, adding a new prospective on their evolutionary meaning. Conversely, in case plants would not be able to operate the release of metallic ions from metal-anthocyanins, the ability to form metal-anthocyanin complexes would be advantageous for anthocyanin-producing species only under condition of metal toxicity, while the same chelation ability would represent a limit when plant cell has to remobilize the metallic ions.

Conclusion

Despite the attractiveness of the hypothesis which involve anthocyanins as part of metal homeostasis, the remobilization of metals form anthocyanin-metal complexes in conditions of transient metal shortage have never been postulated or found yet. Given (i) the demonstrated ability of anthocyanins to chelate metal ions; (ii) the great benefits that this bind confers to the plant under conditions of metal excess, the hypothesis that anthocyanins can interact with (and be part of) a complex network of metal homeostasis worth to be tested. The impact of these findings might add new insights in understanding the ecological and evolutionary role(s) of this intriguing class of pigments widely diffuse in *Plantae*.

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Ethics

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References

Agati, G., P. Matteini, A. Goti and M. Tattini, 2007. Chloroplast-located flavonoids can scavenge singlet oxygen. New Phytol., 174: 77-89. DOI: 10.1111/j.1469-8137.2007.01986.x

Alfenito, M.R., E. Souer, C.D. Goodman, R. Mol and J. Koes *et al.*, 1998. Functional complementation of anthocyanin sequestration in the vacuole by widely divergent glutathione S-transferases. Plant Cell, 10: 1135-1149. DOI: 10.1105/tpc.10.7.1135

Andersen, Ø.M. and M. Jordhein, 2006. The Anthocyanin. In: Flavonoids: Chemistry, Biochemistry and Applications. Andersen, Ø.M. and K.P. Markham (Eds.), CRC Press, Boca Raton, FL, ISBN-10: 142003944X, pp: 451-471.

Boudet, A.M., 2012. Polyphenols: From Plant Adaptation to Useful Chemical Resources. In: Recent Advances in Polyphenol Research, Cheynier, V., P. Sarni-Manchado and S. Quideau (Eds.), John Wiley and Sons, Oxford, ISBN-10: 1444337467, pp: 18-53.

Briat, J.F., C. Curie and F. Gaymard, 2007. Iron utilization and metabolism in plants. Curr. Opin. Plant Biol., 10: 276-282. DOI: 10.1016/j.pbi.2007.04.003

Brunetti, P., L. Zanella, A. Proia, A. De Paolis and G. Falasca *et al.*, 2011. Cadmium tolerance and phytochelatin content of Arabidopsis seedlings over-expressing the phytochelatin synthase gene AtPCS1. J. Exp. Bot., 62: 5509-5519. DOI: 10.1093/jxb/err228

Buchweitz, M., G. Guidi, R. Carle, D.R. Kammerer and H. Schulz, 2012. Systematic investigations of anthocyanin-metal interactions by Raman spectroscopy. J. Raman Spectrosc., 43: 2001-2007. DOI: 10.1002/jrs.4123

Chalker-Scott, L., 1999. Environmental significance of anthocyanins in plant stress responses. J. Raman Spectroscopy, 70: 1-9. DOI: 10.1111/j.1751-1097.1999.tb01944.x

Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie, 88: 1707-1719. DOI: 10.1016/j.biochi.2006.07.003

Elhabiri, M., P. Figueiredo, K. Toki, N. Saito and R. Brouillard, 1997. Anthocyanin-aluminium and–gallium complexes in aqueoussolution. J. Chem. Soc. Perkin Trans., 2: 355-362. DOI: 10.1039/A603851D

Gould, K.S., 2010. Muriel Wheldale Onslow and the Rediscovery of Anthocyanin Function in Plants. In: Recent Advances in Polyphenol Research, Santos-Buelga, C., M.T. Escribano-Bailon and V. Lattanzio (Eds.), John Wiley and Sons, Oxford, ISBN-10: 1444337467, pp: 206-224.

Gould, K.S., K. Davies and C. Winefield, 2009. Anthocyanins: Biosynthesis, Functions and Applications 1st Edn., Springer Science and Business Media, New York, ISBN-10: 0387773355, pp: 336.

Gould, K.S., J. McKelvie and K.R. Markham, 2002. Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. Plant Cell Environ., 25: 1261-1269. DOI: 10.1046/j.1365-3040.2002.00905.x

Guidi, L., C. Penella and M. Landi, 2015. Anthocyanins in Mediterranean Diet: Common and Innovative Sources. In: Handbook of Anthocyanins: Food Sources, Chemical Application and Health Benefit, Warner, L.H. (Ed.), Nova Science Pub Incorporated, New York, ISBN-10: 1633217620, pp: 1-50.

Hale, K.L., S.P. McGrath, E. Lombi, S.M. Stack and N. Terry et al., 2001. Molybdenum sequestration in brassica species. A role for anthocyanins? Plant Physiol., 126: 1391-1402.
DOI: 10.1104/pp.126.4.1391

Hale, L., H.A. Tufan, I.J. Pickering, G.N. George and N. Terry et al., 2002. Anthocyanins facilitate tungsten accumulation in Brassica. Physiol. Plantarum, 116: 351-358. DOI: 10.1034/j.1399-3054.2002.1160310.x

Harborne, J.B. and C.A. Williams, 2000. Advances in flavonoid research since 1992. Phytochemistry, 55: 481-504. DOI: 10.1016/S0031-9422(00)00235-1

Hayashi, K., 1957. Fortschritte der anthocyan forschung in Japan mit besonderer Berucksichti gung der papierchromatographischen Methoden. Pharmazie, 12: 245-249.

Hayashi, K., Y. Abe and S. Mitsui, 1958. Blue anthocyanin from the flowers of commelina, the crystallisation and some properties thereof studies on anthocyanins XXX. Proc. Jpn. Ac., 34: 373-378.
DOI: 10.2183/pjab1945.34.373

Hermans, C., J.P. Hammond, P.J. White and N. Verbruggen, 2006. How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci., 11: 610-617.
DOI: 10.1016/j.tplants.2006.10.007

Hughes, N.M. and S. Lev-Yadun, 2015. Red/purple leaf margin coloration: Potential ecological and physiological functions. Environ. Exp. Bot., 119: 27-39. DOI: 10.1016/j.envexpbot.2015.05.015

Kenrick, P. and P.R. Crane, 1997. The origin and early evolution of plants on land. Nature, 389: 33-39.
DOI: 10.1038/37918

Kondo, T., M. Ueda, H. Tamura, K. Yoshida and M. Isobe et al., 1994. Composition of protocyanin, a self-assembled supramolecular pigment from the blue cornflower, Centaurea cyanus. Angew Chem. Int. Edit., 33: 978-979.
DOI: 10.1002/anie.199409781

Kondo, T., K. Yoshida, A. Nakagawa, T. Kawai and H. Tamura et al., 1992. Structural basis of blue-colour development in flower petals from Commelina communis. Nature, 358: 515-518.
DOI: 10.1038/358515a0

Kovinich, N., G. Kayanja, A. Chanoca, K. Riedl and M.S. Otegui et al., 2014. Not all anthocyanins are born equal: Distinct patterns induced by stress in Arabidopsis. Planta, 240: 931-940.
DOI: 10.1007/s00425-014-2079-1

Kramer, U., I.N. Talke and M. Hanikenne, 2007. Transition metal transport. FEBS Lett., 581: 2263-2272.
DOI: 10.1016/j.febslet.2007.04.010

Kytridis, V.P. and Y. Manetas, 2006. Mesophyll versus epidermal anthocyanins as potential in vivo antioxidants: Evidence linking the putative antioxidant role to the proximity of oxy-radical source. J. Exp. Bot., 57: 2203-2210.
DOI: 10.1093/jxb/erj185

Landi, M., L. Guidi, A. Pardossi, M. Tattini and K.S. Gould, 2014. Photoprotection by foliar anthocyanins mitigates effects of boron toxicity in sweet basil (Ocimum basilicum). Planta, 240: 941-953.
DOI: 10.1007/s00425-014-2087-1

Landi, M., M. Tattini and K.S. Gould, 2015. Multiple functional roles of anthocyanins in plant-environment interactions. Environ. Exp. Bot., 119: 4-17. DOI: 10.1016/j.envexpbot.2015.05.012

Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd Edn., Elsevier, Amsterdam, ISBN-10: 0080571875, pp: 889.

Menzies, I.J., L.W. Youard, J.M. Lord, K.L. Carpenter and J.W. van Klink et al., 2015. Leaf colour polymorphisms: A balance between plant defence and photosynthesis. J. Ecol.
DOI: 10.1111/1365-2745.12494

Nanda-Kumar, P.B.A., V. Dushenkov, H. Motto and I. Raskin, 1995. Phytoextraction: The use of plants to remove heavy metals from soils. Environ. Sci. Technol., 29: 1232-1238.
DOI: 10.1021/es00005a014

Nigel, C.V. and R.J. Grayer, 2008. Flavonoids and their glycosides, including anthocyanins. Nat. Prod. Rep., 25: 555-611. DOI: 10.1039/B718040N

Nikiforova, V.J., B. Gakiere, S. Kempa, M. Adamik and L. Willmitzer et al., 2004. Towards dissecting nutrient metabolism in plants: A systems biology case study on sulphur metabolism. J. Exp. Bot., 55: 1861-1870. DOI: 10.1093/jxb/erh177

Pardossi, A., M. Romani, G. Carmassi, L. Guidi and M. Landi et al., 2015. Boron accumulation and tolerance in sweet basil (Ocimum basilicum L.) with green or purple leaves. Plant Soil, 395: 375-389.
DOI: 10.1007/s11104-015-2571-9

Peng, M., D. Hudson, A. Schofield, R. Tsao and R. Yang et al., 2008. Adaptation of Arabidopsis to nitrogen limitation involves induction of anthocyanin synthesis which is controlled by the NLA gene. J. Exp. Bot., 59: 2933-2944. DOI: 10.1093/jxb/ern148

Rozema, J., L.O. Björn, J.F. Bornman, A. Gaberščik and D.P. Häder et al., 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems-an experimental and functional analysis of the evolution of UV-B absorbing compounds. J. Photochem. Photobiol., 66: 2-12.
DOI: 10.1016/S1011-1344(01)00269-X

Shibata, K., Y. Shibata and I. Kasiwagi, 1919. Studies on anthocyanins: Colour variation in anthocyanins. J. Am. Chem. Soc., 41: 208-220. DOI: 10.1021/ja01459a008

Shoji, K., N. Miki, N. Nakajima, K. Momonoi and C. Kato *et al*., 2007. Perianth bottom-specific blue color development in Tulip cv. Murasakizuisho requires ferric ions. Plant Cell Physiol., 48: 243-251. DOI: 10.1093/pcp/pcm079

Somaatmadja, D., J.J. Powers and M.H. Hamdy, 2006. Anthocyanins. VI. Chelation studies on anthocyanins and other related compounds. J. Food Sci., 29: 655-660. DOI: 10.1111/j.1365-2621.1964.tb00426.x

Stafford, A.H., 1991. Flavonoid evolution: An enzymic approach. Plant Physiol., 96: 680-685. DOI: 10.1104/pp.96.3.680

Stewart, A.J., W. Chapman, G.I. Jenkins, I. Graham and T. Martin *et al*., 2001. The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. Plant Cell Environ., 24: 1189-1197. DOI: 10.1046/j.1365-3040.2001.00768.x

Steyn, W.J., S.J.E. Wand, D.M. Holcroft and G. Jacobs, 2002. Anthocyanins in vegetative tissues: A proposed unified function in photoprotection. New Phytol., 155: 349-361. DOI: 10.1046/j.1469-8137.2002.00482.x

Strack, D., 1997. Phenolic Metabolism. In: Plant Biochemistry, Dei, P.M. and J.B. Harborne (Eds.), Academic Press, San Diego, CA, ISBN-10: 080525725, pp: 387-416.

Takeda, K. and K. Hayashi, 1977. Metallo anthocyanins. I. Reconstruction of commelinin from its components, awobanin, flavocommelin and magnesium. Proc. Jpn. Acad., 53: 1-5. DOI: 10.2183/pjab.53.1

Tattini, M., M. Landi, C. Brunetti, C. Giordano and D. Remorini *et al*., 2014. Epidermal coumaroyl anthocyanins protect sweet basil against excess light stress: Multiple consequences of light attenuation. Physiol. Plantarum, 152: 585-598. DOI: 10.2183/pjab.53.1

Waldron, K.J., J.C. Rutherford, D. Ford and N.J. Robinson, 2009. Metalloproteins and metal sensing. Nature, 460: 823-830. DOI: 10.1038/nature08300

Willstätter, R. and A.E. Everest, 1913. Über den Farbstoff der Kornblume. Justus Liebigs Ann. Chem., 408: 147-162.

Willstätter, R. and T.J. Nolan, 1915. Untersuchungen über die Anthocyane. II. Über den Farbstoff der Rose. Justus Liebigs Ann. Chem., 408: 1-14.

Yadav, S.K., 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S. Afr. J. Bot., 76: 167-179. DOI: 10.1016/j.sajb.2009.10.007

Yoshida, K., M. Mihoko and T. Kondo, 2009. Blue flower color development by anthocyanins: From chemical structure to cell physiology. Nat. Prod. Rep., 26: 857-964. PMID: 19554240