Multiple functional roles of anthocyanins in plant-environment interactions

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ABSTRACT

Almost a century after Muriel Wheldale's landmark publication, "The Anthocyanin Pigments of Plants" (Wheldale, 1916), much about anthocyanin function in plants remains to be elucidated, even though the environmental drivers and biosynthetic pathways leading to anthocyanin production are well established. That anthocyanins may protect leaves in plants facing biotic or abiotic stressors, an idea dating back to Pringsheim (1879), is arguably the most widely accepted function for foliar anthocyanins, although the mechanism(s) by which they could mitigate effects of stress remains heatedly debated. In addition to the most frequently discussed putative roles of anthocyanins as antioxidants and sunscreens, other less-explored possibilities might equally serve to ameliorate plant function under, for example, under conditions of mineral imbalance. In particular: (i) anthocyanins may serve as metal-chelating agents under conditions of excess edaphic metal ions and (ii) anthocyanin accumulation might delay foliar senescence, which is otherwise usually accelerated in plants growing under macronutrient deficiency. The latter mechanism might be particularly advantageous to prolong plant survival and increase the possibility of reproductive success.

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1. Introduction

The word 'anthocyanin' (Greek: *anthos*, flower and *kyanos*, blue) dates back to 1835 when Marquart first coined this term in the paper, "*Die Farben der Bluthen,eine chemisch-physiologische Abhandlung*" (Marquart, 1835). Prior to that, scientists referred to these pigments as cyanophylls, chrythrophylls or cyanins, as explained in the first book dedicated to anthocyanins: "*The Anthocyanin Pigments of Plants*", written by Muriel Wheldale in 1916. Willstätter and Everest (1913), Willstätter and Nolan (1915), and Shibata et al. (1919), pioneers in anthocyanin research, are credited with conducting the first experiments on the pH-dependent change of anthocyanin colors using *Centaurea cyanus* flowers. However, Boyle (1664) in his paper "*Experiments and Considerations Touching Colors*" also offered clear evidence of color change – from intense purple to green – of the "*syrup of violet*" when exposed to "*acid liquor*" (see Wheldale, 1916). Shibata et al.

(1919) also described for the first time that the development *C. cyanus* petal color was attributable to the conjugation of anthocyanins with metal ions (details given in Section 3).

Anthocyanins, one of the most conspicuous classes of flavonoids together with proanthocyanidins and flavonols, are important plant pigments responsible for the red, pink, purple, and blue colors in plants (Grotewold, 2006). Anthocyanins accumulate in the vacuoles of a wide range of cells and tissues in both vegetative and reproductive organs (Hatier and Gould, 2009). From the Antarctic purple-colored leafy liverwort *Cephaloziella exiliflora*, to the breathtaking North American woodland trees (painted by intense autumn-red leaves in species such as *Acer rubrum*), the black foliage of *Ophiopogon planiscapus* 'Nigrescens' (native to Japan), the intense purple-leafed *Prunus cerasifera* (widely dispersed in the Mediterranean basin and Southwest Asia), and the red-pigmented leaf margin of *Pseudowintera colorata* (from New Zealand), anthocyanic foliage is widespread throughout the plant kingdom and occurs all around the world. Nonetheless, although it is clear that one or more selective pressures have driven the evolution of anthocyanin metabolism in disparate vegetative organs (Serna and Martin, 2006; Rausher, 2006), the specific driver (s) that lead to the production of these pigments remains unresolved (Hatier and Gould, 2009).

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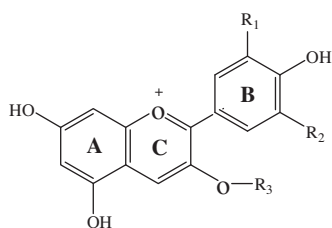
Antocyanin	R_1	R_2	λ max (nm)	
			R_3=H	R_3=glucoside
Pelargonidin	H	H	520	516
Cyanidin	OH	H	535	530
Peonidin	OCH$_3$	H	532	528
Delphinidin	OH	OH	546	541
Petunidin	OH	OCH$_3$	543	540
Malvidin	OCH$_3$	OCH$_3$	542	538

Fig. 1. Flavylium cation and the most abundant anthocyanidins in nature; their maximal absorbance (λ_{max}; nm) in acidified methanol (0.01% HCl).

Anthocyanins are di- or tri-hydroxy B-ring-substituted flavonoids containing a flavylium cation (Fig. 1) which, owing to its conjugated double bonds, absorbs visible light with a peak in the 500–550 nm waveband. The wide range of anthocyanin-derived colors depends on the degree of hydroxylation and the number and/or type of substituted groups. Anthocyanin aglycones, *i.e.*, anthocyanidins, are usually penta-(3, 5, 7, 3′, 4′) or hexa-(3, 5, 7, 3′, 4′, 5′)hydroxy-substituted compounds (Cavalcanti et al., 2011). Although 17 anthocyanidins have been isolated to date, most of these are found only in reproductive structures. Six aglycones (pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin) have been identified in vegetative organs (Andersen and Jordheim, 2006).

Anthocyanins are synthesized in a branch of the flavonoid pathway, which is also involved in the biosynthesis of isoflavonoids and flavonols (reviewed by Winkel, 2006). Enzymes involved in anthocyanin biosynthesis are localized on the cytosolic side of the smooth endoplasmic reticulum (ER), organized into a multi-enzyme complex also known as "flavonoid metabolon" (Winkel, 2004; Saslowsky et al., 2005). Anthocyanins are transported from the endoplasmic reticulum to the cell vacuole through MATE (multidrug and toxic compound extrusion) and ABC (ATP-binding cassette) membrane transporters and/or vesicles (reviewed by Kitamura, 2006; Grotewold, 2006; Zhao and Dixon, 2010; Petrussa et al., 2013). MATE are secondary transporters that use an electrochemical gradient (usually H$^+$ in plants) as the driving force to transport various substrates, including anthocyanins, across membranes (Verweij et al., 2008). ABC transporters (in particular from ABCC subfamily, also known as multidrug resistance-associated protein; MRP/ABCC) are also capable of direct translocation of many substrates, including anthocyanins, after their conjugation with glutathione (GSH) by a reaction catalyzed by glutathione S-transferases (GST) (Alfenito et al., 1998; Zhao and Dixon, 2010; Martinoia et al., 2012; Francisco et al., 2013). Recently, Francisco et al. (2013) found that grapevine ABCC1, when expressed in grape berries, mediates the GSH-dependent vacuolar transport of anthocyanidin-3-O-glucosides. The authors found a 1:1 stochiometry for the transport of GSH and anthocyanins, but that GSH alone is not recognized as a substrate by ABCC1, suggesting that GSH and anthocyanins are brought together in the enzyme cavity *via* an unknown mechanism to facilitate their transport. Vesicle-mediated transport has been, for the most part, investigated for the vacuolar accumulation of anthocyanins, in which proanthocyanidin precursors synthesized at the ER

membranes enter the ER lumen, and then ER-derived vesicles containing the precursors are transported to the vacuole, where they fuse with the tonoplast membrane and empty their contents inside the vacuole (for reviews, see Zhao and Dixon, 2009; Zhao et al., 2010). The transport and accumulation of anthocyanins into the vacuole may also constitute a limiting step in anthocyanin biosynthesis (Zhao and Dixon, 2009).

Some flavonoids can move from their site of synthesis to other parts of the plant (Saslowsky and Winkel-Shirley, 2001; Grotewold, 2004; Buer et al., 2007). If this were possible for anthocyanins, it could allow, for example, accumulation of the pigment in subterranean organs that grow in the dark, such as purple potato tubers (Mano et al., 2007) or red rhizomes (Neufeld et al., 2011). However, long distance transport of anthocyanin has never been demonstrated. Indeed, root tissues often have all the enzymes required for anthocyanin biosynthesis (Maloney et al., 2014). The roots of *Ginura bicolor*, for example, can accumulate anthocyanins, but only when they are exposed to light (Shimizu et al., 2011), since the expression of genes encoding for chalcone synthase (CHS) has an obligate light requirement (Saslowsky and Winkel-Shirley, 2001; Buer and Muday, 2004; Mano et al., 2007). It has been convincingly shown that precursors of flavonols (*e.g.*, naringenin) can be transported from shoots to roots (Buer et al., 2007). Thus, it is possible that anthocyanin precursors, rather than anthocyanins *per se*, are synthesized in the shoot and then transported to organs that grow in the dark. Notably, some GSTs have been recently hypothesized to be involved in the long-distance transport system (Zhao et al., 2010). Nevertheless, mechanisms of anthocyanin transport remain poorly understood and warrant further investigation.

Because anthocyanins occur so commonly across disparate vegetative organs, there has been concerted research effort to understand their functional significance, particularly in plants that experience abiotic stressors (Chalker-Scott 1999; Hoch et al., 2001; Steyn et al., 2002; Close and Beadle 2003; Gould, 2004; Manetas, 2006; Gould et al., 2009; Archetti, 2009a; Hughes, 2011; Tonelli et al., 2015). Nonetheless, after well over a century of research and despite a comprehensive knowledge on the regulation of anthocyanin biosynthesis (Winkel, 2006), their functional roles in plant–environment interactions remain highly contested (for a review see Gould et al., 2009). Indeed, different species apparently derive quite different benefits from the very same anthocyanin moiety (Hatier and Gould, 2009), thus making it difficult to provide a unified explanation for their functional roles. Here, we describe recent evidence for four putative functions of anthocyanins as (1) sunscreens and antioxidants, (2) mediators of reactive oxygen species (ROS)-induced signalling cascades (Hatier and Gould, 2008, 2009), (3) chelating agents for metals and/or metalloids (Hale et al., 2001, 2002; Nissim-Levi et al., 2003; Glińska et al., 2007; Arreola et al., 2008; Posmyk et al., 2009; Landi et al., 2014), and (4) delayers of leaf senescence, especially in plants growing under nutrient deficiency (Peng et al., 2007a,b, 2008).

2. The photoprotective functions of foliar anthocyanins: sunscreens, antioxidants or both?

Anthocyanin biosynthesis is just one component of a suite of metabolic adjustments made in response to high sunlight irradiance (Tattini et al., 2005, 2014). Since the constitutive morpho-anatomical traits of a leaf can, to some degree, control the extent to which sunlight affects its biochemistry, the significance of possible photoprotective functions of anthocyanins may vary substantially across species (Hughes and Smith, 2007; Kytridis et al., 2008).