Foliar anthocyanins as modulators of stress signals

Possible functions of foliar anthocyanins have in recent years attracted heated scientific debate. Anthocyanins, the water-soluble pigments that supply many of the reds, purples, and blues to plant organs, are prevalent throughout all phyla of the plant kingdom. The functional role of anthocyanins in reproductive structures seems clear—to attract animal vectors for pollination and/or seed dispersal. However, the selective pressure that has driven the evolution of anthocyanins in vegetative structures such as leaves remains far from obvious. Many plant physiologists champion the hypothesis that foliar anthocyanins serve to protect plant cells from the adverse effects of abiotic stressors; these would include strong light, UV-B radiation, temperature extremes, heavy metals, and drought (Chalker-Scott, 1999; Steyn et al., 2002; Gould and Lister, 2006). In contrast, growing numbers of predominantly theoretical papers dismiss or largely ignore the physiological evidence, asserting instead that the primary function of these pigments is to assist in plant defence; putative roles include aposematic signalling, mimicry of unpalatable foliage, camouflage, or the undermining of crypsis of herbivorous insects (Archetti, 2000; Hamilton and Brown, 2001; Lev-Yadun et al., 2004; Lev-Yadun and Gould, 2007; Rolshausen and Schaefer, 2007). This ever-gaping dichotomy has led some workers to call for research that addresses both ecological and physiological functions to determine which best explains the evolution of non-green leaves in nature (Schaefer and Wilkinson, 2004).

Of course, the selective pressures that led to the evolution of red leaves do not necessarily correlate to current function or advantage. Schaefer and Rolshausen (2006) presented a particularly compelling argument for this in what they termed the ‘defence indication hypothesis’. Accordingly, the primary driving force that led to anthocyanin pigments was considered to be an abiotic stressor such as strong light. However, because anthocyanins are end products of a biosynthetic pathway that also produces herbivore deterrents and antifeedants such as condensed tannins, then the red pigments would correlate to, and be indicative of, defensive strength. Thus, anthocyanins in a leaf might simultaneously protect chloroplasts from excess quanta and repel opportunistic herbivores. Alternatively, there may be a unifying mechanism by which anthocyanins protect leaves both from abiotic stressors and from colonising insects. This is achievable, we suggest, if anthocyanins were to function as regulators of the signal transduction cascades involved in the amelioration of stress responses. As explained below, anthocyanins might accomplish this (i) by protecting antioxidant enzymes, (ii) by scavenging free radicals and other reactive oxygen species (ROS) directly, and/or (iii) through interactions with other molecules in the signalling pathways (Fig. 1). Almost all stressors, including abiotic stimuli and injuries from herbivorous insects, lead to a transient surge in ROS such as superoxide and hydrogen peroxide (Mithofer et al., 2004). Once considered only to be deleterious to plant function, ROS are now believed to play crucial roles as signalling molecules. For example, H$_2$O$_2$ can initiate protein kinase cascades that lead to the activation or suppression of transcription factors, ultimately regulating the expression of genes involved in stress responses (Laloi et al., 2004; Mullineaux et al., 2006; Gechev et al., 2006). Plants must retain sufficient low levels of the different types of ROS for signalling purposes, yet neutralise the excess ROS to avoid oxidative damage. This fine-tuning is achieved by the coordinated activities of antioxidant enzymes, which scavenge ROS with extreme efficiency and are undoubtedly key players in the modulation of signalling cascades (Foyer and Noctor, 2005; Mittler et al., 2004).

Under certain conditions, however, antioxidant enzymes are inactivated. The enzymes may simply saturate with substrate, and then require metabolic processing to restore their potency (Asada, 2006). In addition, supernumerary ROS, such as H$_2$O$_2$, or the highly toxic hydroxyl radical, can cause irreversible damage to the protein structures of key antioxidant enzymes (Hearn et al., 1999). The potential for damage may be exacerbated when plants experience a combination of stressors, such as strong light and low temperatures; such conditions promote ROS generation from chloroplasts, but because enzymes are usually less active at lower temperatures, the ROS-scavenging capacities are reduced. This can lead to the accumulation of H$_2$O$_2$ to levels that inactivate the peroxidases, catalase, and superoxide dismutase (Jahnke et al., 1991; Wise, 1995; Casano et al., 1997; Streb et al., 1997). For stress signalling cascades to function properly, the enzymatic antioxidants need to be protected from a superabundance of ROS.

It is probably no coincidence that the very conditions that can lead to the inactivation of antioxidant enzymes can also induce the biosynthesis of foliar anthocyanins. These red pigments absorb a significant portion of light energy (Hatrer and Gould, 2007), which, by reducing the quantum load on chloroplasts, diminishes rates of production of ROS from photosynthetic electron transport carriers (Neill and Gould, 2003). Since chloroplasts are a major source of ROS in plants under stress (Asada, 2006; Mullineaux et al., 2006), the light-screening effects of anthocyanins would both spare antioxidant enzymes, and moderate the amplitude of ROS-driven stress-signalling cascades. The one-time investment in the production of a permanent light filter by anthocyanins would likely be energetically less costly than repeated reactivation of spent antioxidant enzymes.

Anthocyanins may interact with stress signals more directly. This has been demonstrated in human tumour cells; aglycones of the two most abundant anthocyanins, cyaniding, and delphinidin, inhibited tumour cell growth by arresting downstream signalling cascades which would otherwise have led to the production of growth factors (Meiers et al., 2001). As potent scavengers of a variety of free radicals, anthocyanins are chemically well suited to modulate the general balance of ROS within a cell (Yamasaki et al., 1996). Anthocyanins reside predominantly inside the plant cell.

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vacuole, which, by virtue of its size and proximity to all organelles, is considered to be an important regulator of ROS metabolism (Mittler et al., 2004). H$_2$O$_2$ in particular is likely to leak into the vacuole during periods of stress; it diffuses rapidly between cell compartments (Gechev et al., 2006; Henzler and Steudle, 2000; Yamasaki, 1997). Vacuolar antioxidants such as the anthocyanins are likely to have a crucial role, especially in restricting the movement of H$_2$O$_2$ within and between plant cells (Gould et al., 2002).

Finally, anthocyanins may act downstream of the ROS signalling pathway, or else be involved in the crosstalk with other response pathways. There is growing support, for example, for interactions between anthocyanins and sucrose. The genes for key enzymes involved in anthocyanin biosynthesis are strongly upregulated by sucrose (Solfanelli et al., 2006), which, along with other soluble sugars, has been implicated in the acquisition of tolerance to environmental stress (Couée et al., 2006; Loret et al., 2005). Sucrose is involved in both ROS-producing and ROS-scavenging metabolic pathways, and probably assists in the regulation of the pro-oxidant and antioxidant balance in plant cells (Couée et al., 2006). However, possible mechanisms by which sucrose, anthocyanins, and ROS interact to contribute to plant function are unknown.

Our ‘signal modulation’ hypothesis for anthocyanin function could apply equally to plant–biotic interactions as to the responses to abiotic stressors. When leaves are wounded by herbivores or, indeed, infected by pathogens, an ensuing burst of ROS triggers a chain of molecular signalling events, mediated by oxylipins, jasmonic acid, ethylene and/or salicylic acid, which ultimately lead to the expression of defence-related genes (Kessler and Baldwin, 2002; Farmer et al., 2003). In wounded red leaves, the constitutive anthocyanins would contribute to the modulation of these signalling responses, both by scavenging ROS directly, and by sparing antioxidant enzymes from potential damaging effects of excess ROS. Green leaves, too, often produce anthocyanic lesions in response to herbivore injury (Costa-Arbulú et al., 2001; Gould et al., 2002). Such lesions are usually highly localised around the site of injury, often no more than a few millimetres in radius from the centre of the wound. It seems unlikely, therefore, that these inducible anthocyanins would protect the entire leaf lamina. They could, however, assist in the protection of areas contiguous to the wound against secondary infections by fungal pathogens, and from reinvasions by herbivores.

Our hypothesis does not preclude additional functions of foliar anthocyanins, such as aposematism or photoprotection. However, as chemical messengers, anthocyanins would belong in the same category as their molecular cousins, the flavones, flavanones, and flavonols, for which there is abundant evidence of activities in diverse signalling processes (Gould and Lister, 2006). A signalling role would not only bridge the chasm between defensive and physiological hypotheses for the presence of anthocyanins in leaves, it could also explain why the pigments accumulate in organs such as roots, which are not exposed to light. Establishing possible relationships between the cellular redox balance and anthocyanin function promises to be an exciting new line of investigation into this intriguing class of plant pigments.

References


Fig. 1. Signal-modulation hypothesis for anthocyanin function in leaves. Anthocyanins reduce production of reactive oxygen species (ROS), increase rates of ROS scavenging, and interact with soluble sugars, thereby protecting antioxidant enzymes and facilitating efficient oxidative signalling following biotic or abiotic stress. Positive (arrows) and negative (T-bars) interactions are shown.


