Invited Review

Environmental Significance of Anthocyanins in Plant Stress Responses

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ABSTRACT

Anthocyanins are water-soluble pigments found in all plant tissues throughout the plant kingdom. Our understanding of anthocyanin biosynthesis and its molecular control has greatly improved in the last decade. The adaptive advantages of anthocyanins, especially in non-reproductive tissues, is much less clear. Anthocyanins often appear transiently at specific developmental stages and may be induced by a number of environmental factors including visible and UV-B radiation, cold temperatures and water stress. The subsequent production and localization of anthocyanins in root, stem and especially leaf tissues may allow the plant to develop resistance to a number of environmental stresses. This article reviews the environmental induction of anthocyanins and their proposed importance in ameliorating environmental stresses induced by visible and UV-B radiation, drought and cold temperatures.

INTRODUCTION

The characteristic red, blue and purple coloration seen in various tissues of a diverse assortment of plants is due to anthocyanins either in part (flowers and fruits) or exclusively (leaves). Anthocyanins are water-soluble pigments derived from flavonoids via the shikimic acid pathway. Anthocyanins may be developmentally transient, appearing only in juvenile or senescing tissues, or they may be permanent. Likewise, they may be environmentally transient, appearing and disappearing with changes in photoperiod, temperature or other signals. The photinduction, signal transduction, gene expression and biosynthetic pathway of anthocyanins have been extensively studied and recently reviewed (1–3). The literature on their environmental induction and subsequent impact on plant survival, especially in regard to non-reproductive tissues, has not been reviewed, however. In terms of adaptive advantage, leaf anthocyanins may be the least understood group of non-photosynthetic pigments in plants.

MORPHOLOGICAL AND DEVELOPMENTAL ASSOCIATIONS

Tissue location
Apart from their widely reported distribution in floral and fruit tissues, anthocyanins are also located in roots, shoots and leaves. Anthocyanins have been reported in the root cap of Impatiens seedlings (4) and in roots under osmotic (5) or toxin (6) stress. Stems may accumulate anthocyanins (7,8), often as a function of juvenility (9–11), osmotic stress (12,13) or cold temperatures (14–17).

Leaves of both gymnosperms (9,18) and angiosperms (17,19–34) accumulate anthocyanins in their tissues. In most cases, anthocyanins are found in or just below the upper epidermis of leaves (18,20,31,33–35). Shade plants accumulate anthocyanins in their lower epidermis, perhaps to assist in light capture (26,27). Notably, anthocyanins have been reported to occur only in the mesophyll layers of other plant species including Mahonia, Viburnum and Rhododendron (24) and the tropical woody genus Syzygium (33).

Juvenile reddening
One of the most striking phenomena associated with anthocyanins is the juvenile reddening exhibited by a variety of plant species. Many herbaceous seedlings accumulate anthocyanins transiently, often as a result of photoinhibition and always within hours or days after germination (5,36–53). Seedlings of woody species seem to require additional environmental cues such as cold temperatures (9,14,18) to accumulate anthocyanins, while their mature counterparts do not demonstrate this ability. Temperate woody perennials with juvenile reddening may show leaf flush in response to cool temperatures (54), while deciduous tropical species produce red leaves in response to drought (21,55). Yet other tropical species produce juvenile anthocyanins in response to as yet unknown signals; light-harvesting, photoprotection and antifungal defenses have been variously proposed (26,27,33,34,56).

INDUCTION OF ANTHOCYANIN BIOSYNTHESIS

Photoinduction
Most research on anthocyanins has focused on their photoinduction by wavelengths in the UV, visible and far-red regions. The importance of a light stimulus is further support-
ed by the dark inhibition of anthocyanin synthesis (9,15,57), although too much radiation in the UVB region inhibits anthocyanin synthesis, probably via DNA damage (29,38,44).

Although photoinduction of anthocyanins has been demonstrated numerous times in both the laboratory and field, the actual photoreceptors responsible have not been clearly identified. In early research, Lindoo and Caldwell (28) theorized that far-red and UVB radiation (and therefore their photoreceptors) acted independently on anthocyanin formation in Rumex patientia. Further research seemed only to increase the murkiness of the debate. In reviewing the widespread phenomena of juvenile anthocyanin production in seedlings, Mohr et al. (48) skirt the issue by stating that "the actual induction wavelength varies among species."

Subsequent studies of various plant species and tissues have divided researchers into two camps: those who assert that anthocyanin synthesis is induced by the UVB photoreceptor (25,37,50,52,53,57–64) and those who argue that induction is through some combination of the UVB photoreceptor, phytochrome and cryptochrome (16,36,40,41,45,46,65–68). Convincing evidence for the first hypothesis is illustrated by Brandt et al. (37), who found a Lycopersicon mutant deficient in phytochrome accumulated anthocyanins via UVB exposure. In UVB exclusion experiments, Krizek et al. (25) found significant decreases in both flavonoid and anthocyanin concentrations of red-leaved lettuce. Ambasht and Agrawal (58) found field-grown Zea mays accumulated anthocyanins following UVB exposure. Conversely, Beggs and Wellmann (36) found some varieties of Z. mays to synthesize anthocyanins in response to far-red, UVB or to no light at all.

In their review, Mol et al. (3) suggest that phytochrome is involved in anthocyanin synthesis, but that another photoreceptor is also involved. They further suggest the existence of two separate anthocyanin pathways under differential regulation. Perhaps the most reasonable hypothesis is that UVB is the only photoinducant of anthocyanins, while their relative amounts are modulated by phytochrome (50,68). Certainly the majority of the published data could be explained by this mechanism. Furthermore, anthocyanin induction might be modified by developmental stage or by environmental cues such as temperature (16).

Cold temperature induction

Induction of anthocyanins by cold temperatures has received less attention than photoinduction, even though the evidence for this process is seen in deciduous plants every fall. Low temperature has been shown to induce anthocyanin synthesis in seedlings of Arabidopsis (69,70), Sorghum (16), Poncirus (17) and Z. mays (39), leaves of Cotinus (54,71) and Pinus (18), 1 year-old twigs of Diospyros (14) and ray parenchyma cells of Fagus sylvatica (72). Christie et al. (39) consider the anthocyanin biosynthetic pathway to involve cor (cold-regulation) genes but observe that very cold temperatures destroy the biosynthetic capability. McKown et al. (73) agree that there is some commonality between anthocyanin biosynthesis and freezing tolerance, in either the synthetic or regulatory pathways leading to both. It should be noted, however, that low temperatures in the absence of either visible light (23) or UVB (71) prevent anthocyanin biosynthesis. As Mol et al. (3) conclude, the mechanism of cold induction of anthocyanins and role of light is not fully understood and they again suggest separate, or perhaps overlapping, pathways.

Osmotic induction

A third body of information exists relative to the osmotic induction of anthocyanins. Studies with cell cultures of various species find anthocyanin accumulation resulting from osmotic stress induced by glucose (74,75), sucrose (76–81) and mannitol (74,75,78,80,82). Dilution stress was also shown to induce anthocyanins in normally pigment-free Petunia cell cultures (83).

Experiments in whole-plant systems have shown similar results. Anthocyanin accumulation induced by saline conditions was seen in Z. mays roots (5), Morus alba leaves (84) and the lower stems of Casuarina equisetifolia seedlings (12). Arabidopsis (85), Terminalia catappa (86) and Hedera helix leaves (11) all accumulated anthocyanins when grown in the presence of various sugars.

Obviously increased osmotica will induce a dehydration strain on plant cells. In related studies, drought stress significantly increased anthocyanin levels in cowpea seedlings (87), resurrection plants (88) and in Cotinus leaves (Oren-Shamir, unpublished data). Flooded Malus and Pyrus trees showed anthocyanin increases in their leaves (89), probably in response to the secondary drought stress imposed upon leaves by depressed root function.

Other induction factors

An assortment of other intrinsic and environmental factors have been linked to anthocyanin induction, accumulation or inhibition (3,46,69). Deficiencies in nitrogen (19,79,80), phosphorus (80,90,91) or both (92) cause anthocyanins to increase, as does exposure to lowered pH (82), methyl jasmonate (93), wounding (94), pathogen infection (95) and fungal elicitors (95). In contrast, Gläßgen et al. (61), Gleitz et al. (62) and Lo and Nicholson (96) noted suppression of anthocyanins in the presence of fungal elicitors. Differential responses to herbicides were also seen in treated seedlings, with alachlor and rimsulfuron stimulating production and trifluralin and fluometuron inhibiting anthocyanin accumulation (97).

The impact of exogenous growth regulators on anthocyanin accumulation is also unclear. Both auxins and/or cytokinins have been shown to induce anthocyanins in cell cultures (80,81) or whole plant systems (98), and others have linked gibberellins to anthocyanin production (3). However, Ronchi et al. (51) concluded that anthocyanin accumulation in Z. mays was instead linked to gibberellin inhibition. Likewise, gibberellins were seen to have no effect on anthocyanins in Protonia leaves (Oren-Shamir and Nissim-Levi, unpublished data). Although others have noted an inhibitory effect of abscisic acid (ABA) (3,4), Fambri et al. (42) suggest that lack of ABA prevents anthocyanin manufacture.

Abbreviations: ABA, abscisic acid; PAL, phenylalanine ammonia-lyase.
ADAPTIVE SIGNIFICANCE OF ANTHOCYANINS

What adaptive advantages do leaf tissues containing high levels of anthocyanin have over those tissues with lower levels? To answer this question, the relative costs and benefits of anthocyanin accumulation must be compared.

Metabolic costs

Anthocyanin synthesis is metabolically expensive, requiring additional modifications of flavonol precursors. Their eventual degradation, such as that seen during maturation of red juvenile leaves of Brachystegia spp. (21), must also use energy. Another potential cost of anthocyanin accumulation is their interference with the light reactions of photosynthesis. Because of their ability to absorb blue light and reflect red wavelengths, anthocyanins in the upper epidermis or mesophyll of leaves could theoretically compete with light harvesting by chlorophyll and carotenoids. Reductions in photosynthetic rates have been noted in red-leaved varieties of Coleus (20) and pepper (99), spring flushing leaves of Brachystegia spp. (21,55) and the red juvenile leaves of several rainforest tree species (33).

Environmental strain reduction—Photoprotection

Because they are photoinduced, researchers surmise that anthocyanins must have a photoprotective function, either against light-induced photooxidation or against UVB damage. While evidence exists to support the former theory, the role of anthocyanins in UVB protection is much less apparent.

In 1984 Drumm-Herrl (40) suggested that anthocyanins protect against damaging levels of light, especially high-energy blue wavelengths that can damage protochlorophyll in developing leaves. Subsequent studies have supported this theory, although proof is far from definitive. Anthocyanin accumulation during dehydration of resurrection plants (Ceratostigmum wimsii and Xeropitys viscosa) is thought to shield these dehydrated plants from photoinhibition (88). Similar shielding is suggested for 1 month-old Pinus banksiana seedlings (18), juvenile leaves of several rainforest species (33) and evergreen leaves of Mahonia, Viburnum and Rhododendron (24).

Whether the same protection is afforded to UVB radiation is questionable. Numerous studies (100) have implicated UVB radiation in having a significant negative impact on plant productivity, particularly in crop plants that have been bred for soft characteristics. In contrast, resistant or tolerant plants adapt to UVB stress either through photorepair of damage or radiation attenuation. This latter strategy includes thickened cuticles (101) and cell walls (102), incorporation of wall phenolics (102) including lignin (103,104) and increased pubescence (105). As Beggs and Wellmann (36) observed, however, anthocyanins do not absorb well in the UVB range nor could they find hard evidence for a role in UVB protection. Others have concurred with this assessment (37,106), adding that anthocyanins often occur in very low concentrations compared to other UVB-absorbing compounds and require a long exposure to UVB to be synthesized (37).

Nonetheless, there is evidence that anthocyanins do prevent UVB damage in some instances. Cell cultures of Centaurea cyanus were apparently protected from UVB-induced DNA damage by anthocyanins (107). Hada et al. (44) associated decreased anthocyanins with increased DNA damage to Sorghum bicolor seedlings irradiated with UVB. Both Arabidopsis (108) and Zea (109) flavonoid-deficient mutants are hypersensitive to UVB. Gitz et al. (43) noted that red cabbage seedlings treated with the phenylalanine ammonia-lyase (PAL) inhibitor 2-amino-indan-2-phosphonic acid were more damaged by UVB radiation than untreated controls. The PAL inhibition, of course, would also reduce other UVB-absorbing phenolics and flavonoids as well as anthocyanins. On a more speculative note, many researchers have noted the presence of anthocyanins in the upper epidermal layer of leaves belonging to a number of different plant species and associate them with UVB protection (20,21,33,34,55,56,110).

In reviewing this evidence, it is important to understand the differential absorption of covalently modified anthocyanins. Trevino et al. (31) noted that anthocyanins esterified with cinnamic acids do absorb UVB radiation. Likewise, Woodall and Stewart (34) mention that anthocyanins acylated with phenolics such as cinnamic acid are able to absorb higher end UVB (310–320 nm), and the absence of these compounds was directly correlated with UVB damage in red cabbage seedlings (43). Some stresses have been shown to increase the methylation (78) or acylation (90) of anthocyanins. Obviously such alterations would impact the absorption spectrum of these compounds. Similarly, absorptive artifacts could occur during improper isolation of anthocyanins, dissociating covalent bonds.

Finally, the importance of flavonoids should not be overlooked in discussions of anthocyanin production and UVB protection. Flavonoids are induced by UVB (29,31,37,61,111) and recognized as strong UVB absorbers (35,40,106). In fact, their absorption of UVB is much stronger than that of anthocyanins. Therein lies a conundrum: if one of the effects of UVB irradiation on plants is to induce UVB-protective pigments, why are anthocyanins produced instead of their colorless flavonoid precursors? It should be emphasized that anthocyanin production represents a conversion of flavonol precursors (37), which themselves are strong UVB absorbers. Perhaps the improved solubility of anthocyanins, which in contrast to other flavonoids are nearly always glycosylated (112), allow them to be stored in the vacuole more efficiently than nonglycosylated flavonoids.

Environmental strain reduction—Cold hardness

Freezing temperatures can inflict mechanical injury on plant cells via ice crystal formation or induce dehydration as liquid water becomes extracellular ice. In nature, cold-exposed tissues take several weeks to winter harden through various mechanisms including the deposition of phenolic-rich compounds such as lignin in their cell walls. These structural changes allow cells to withstand physical damage from ice formation in extracellular spaces or on epidermal surfaces. Unlike mature tissues, expanding leaves cannot cold harden by lignifying their cell walls. Many plants avoid freeze damage to sensitive tissues through water supercooling as low
as -41°C before freezing (113). Such supercooling is often induced by increasing solute levels and has been associated with xylem ray parenchyma cells, dormant flower buds (114) and leaf tissues (115). Anthocyanin accumulation by epidermal cells in these latter tissues would decrease the osmotic potential of the cell and delay freezing via surf ace nucleators, thus protecting the leaves from late spring frosts.

Decades ago, Parker (30) linked anthocyanin appearance and disappearance to cold hardness in Hedera helix leaves. Further work (116) refuted these results, demonstrating no correlation between hardness and anthocyanin levels in H. helix. It should be noted, however, that these latter experiments were conducted in a greenhouse and therefore the results might not be indicative of field conditions (i.e. natural UVB levels). Parker (30), on the other hand, used field-grown leaf tissues.

More recently, Singh et al. (7) studied anthocyanin content and its relevance to cold hardness of field-grown chickpea (Cicer arietinum). They concluded there was no association between stem anthocyanins and cold hardness but did not report these data in the article. Furthermore, because this was not a controlled experiment (e.g. outdoor temperature fluctuations), they would not be able to assess small, but significant, differences in cold hardness among genetic lines.

The induction of anthocyanins by chilling temperatures does suggest a protective function, and some studies are supportive of this idea. McKown et al. (73) suggest some commonality between anthocyanin biosynthesis and freezing tolerance, as four Arabidopsis mutants deficient in freezing tolerance were unable to accumulate anthocyanins. Autumn induction of anthocyanins is widely known and occurs in tandem with the onset of dormancy and cold hardness in many woody plants. Winter-hardy tissues containing high levels of anthocyanins (14,15,71,117) generally decrease or lose these pigments the following spring. Northern ecotypes of Populus trichocarpa, which presumably survive colder winters than their southern counterparts, accumulate more anthocyanins than southern ecotypes with decreasing photoperiod (118). Krol et al. (18) believe anthocyanins protect the mesophyll of young Pinus seedlings from low-temperature photoinhibition. Anthocyanin-rich species such as Pho tinia have extended growing periods compared to other ornamental shrubs (119), perhaps as a result of increased tolerance of cool temperatures. A preliminary study of cold hardness of green, UV-shielded and red, UV-exposed Cotinus leaves indicates that UV-exposure likewise increases the cold hardness of this species (Chalker-Scott, unpublished data).

Any purported mechanism by which anthocyanins could enhance frost hardness remains unclear. One hypothesis focuses on their ability to raise leaf temperature (120,121). This theory, however, has not been well documented (18,26) and requires further investigation. A more logical explanation might involve cold hardness induction via osmotic control. Following a low-temperature exposure (5-10°C), I believe tissues will immediately show a small but significant increase in hardness. The mechanism of this increase in frost hardness (seen during fall and spring) is osmotic—more solutes (e.g. anthocyanins) in the vacuole mean water freezes at a lower temperature. This small increase in hardness would be enough to protect young tissues from frost damage in late spring. In particular, the accumulation of anthocyanins in epidermal vacuoles would prevent their freezing, especially from leaf surface nucleators. This phenomenon would also protect deciduous leaves from early fall frosts—a physiologically important time in which to mobilize substances for winter storage. Because this mobilization includes sugar transfer, anthocyanins might also facilitate this process because they exist almost exclusively as glycosides (112). Perennial tissues then show a second, more significant increase in cold hardness (seen during the winter) several weeks postexposure that may or may not be related to anthocyanins.

An osmotically induced increase in cold hardness could provide cross-resistance to other stresses, particularly drought. During winter freezes this could be particularly important in protecting sensitive parenchyma cells in the mesophyll or xylem rays of woody perennials from freeze-induced dehydration.

Apart from the problems of ice formation in leaf tissues, cold temperatures also decrease saturation levels of membrane lipids. Membranes with more polyunsaturated fatty acids are more sensitive to UVB damage because they are readily oxidized by radicals formed by UVB (122). Radicals are also longer-lived at lower temperatures, increasing the likelihood of membrane damage. Thus, epidermal anthocyanins are dually protective in preventing damage caused, directly or indirectly, by cold temperatures and UVB.

Environmental strain reduction—Drought resistance

Like freezing stress, drought induces water migration from cells resulting in dehydration and eventual plasmolysis. Plant cells that survive drought conditions generally have extremely thick cuticles (123) and other structural modifications. Tissues that are developmentally unable to synthesize structurally protective compounds in their cell walls (like juvenile leaves) often increase vacuolar solutes to decrease leaf osmotic potential, allowing the plant to remain turgid under low water potential conditions.

Given the induction of anthocyanins by osmotic stress, it is not surprising to find that plant tissues containing anthocyanins are often resistant to drought stress. Often the drought resistance is causatively linked to anthocyanin content by the researchers, but such coincidental information is common in the literature. For instance, the 'Pretty Purple' cultivar of pepper is more resistant to water stress than related green cultivars (99). Ornamental shrubs with high levels of anthocyanins, such as Cotinus and Photinia, tend to be more tolerant to drought conditions (119,124-126).

More direct connections have been made both in field and laboratory settings. Decades ago anthocyanin formation in Populus shoots was associated with drought resistance (13). Resurrection plants, which show extraordinary tolerance for dehydration, accumulate three to four times more anthocyanins during dehydration than in their fully hydrated state (88). Deciduous tropical trees of the genus Brachystegia produce spring flushing leaves rich in anthocyanins 2 months prior to the rainy season; these leaves have been shown to have lower stomatal conductances than the rainy season, green leaves (21,55). Anthocyanin-containing evergreen
leaves of *Mahonia, Viburnum* and *Rhododendron* generally had a lower water content than their green counterparts (24). Like these perennials, maize seedlings with induced anthocyanins also show similar tolerances to drought (51).

Cross-resistance to drought can also be induced by exposure to UVB. Nogué et al. (111) reported that UVB exposure delayed and reduced the severity of drought stress in *Pisum sativum*. Flavonoids and anthocyanins were measured, both increasing significantly and synergistically as a function of both drought and UVB; similar results were also seen in cowpea (87). In another study of cross-resistance, Petropoulou et al. (127) reported that UVB alleviated drought stress in *Pinus pinea* and *Pinus halepensis*. Although anthocyanins were not specifically measured in this study, *Pinus* species are known to produce anthocyanins under UVB exposure (64).

It is interesting to note that this cross-resistance can also occur in the reverse, with the concomitant accumulation of anthocyanins. Water stress helped soybeans avoid additional stress from UVB (110,128), increasing the level of anthocyanins and other UV-absorbing compounds in leaf tissue by 23% (110). Sullivan and Teramura (128) also note that drought may reduce plant phosphorous levels, the deficiency of which had previously been linked to UVB resistance. Coincidentally, one of the classic symptoms of phosphorous deficiency is accumulation of anthocyanins in the leaves.

Drought avoidance is generally seen in plants that can decrease their leaves' osmotic potential to the point where evaporationspiration is minimized. Anthocyanin concentration will *de facto* decrease the osmotic potential (i.e., make it more negative) of leaves, decreasing the leaf water potential and perhaps contributing to the decreased stomatal conductance seen in flushing leaves of *Brachystegia spiciformis* (21) and drought stress resistance in *Photinia × fraseri* 'Red Top' (124).

**Environmental strain reduction—Antioxidative abilities**

The antioxidant capabilities of anthocyanins have only recently been investigated. Grace et al. (22) noticed that levels of antioxidant enzymes such as superoxide dismutase showed either slight increases or actually decreased in conjunction with photoinduced anthocyanin production in *Mahonia repens*. Ozone exposure was found to induce anthocyanins (117); it is possible that anthocyanins could have antioxidant functions in the presence of this powerful oxidizer. A similar speculation is provided by Sherwin and Farrant (88), who noted significant anthocyanin production during dehydration of resurrection plants. Ishii et al. (129) found the antioxidative ability of isolated anthocyanins to be greater than that of α-tocopherol. Perhaps the most convincing evidence comes from Tsuda et al. (130,131) in their work with liposomes, micromes and membrane systems. In all systems they noted that introduced anthocyanins scavenged oxygen radicals and inhibited lipid peroxidation.

**Environmental strain reduction—Antifungal/antibiotic capabilities**

Only speculation seems to exist on the role of anthocyanins as antifungal or antibiotic agents. Because fungus-growing leaf cutter ants avoid high levels of anthocyanins, Coley and Aide (132) propose an antifungal role based on research by Powers (133) and others. Similarly, Tuhoy and Choinski (55) hypothesize that anthocyanin-containing, spring flushing foliage of *Brachystegia* are antifungal, even though these leaves are produced prior to the wet season. Insects may be deterred by anthocyanins in tomatoes (19), but again this is speculation based on previous work (134) and has been actively discounted in other studies (26,132).

**Cross-resistance**

Many authors have commented on the similarities among the physiological and morphological responses to various abiotic stresses including UVB, cold and drought. Previous research by the author (135,136) and others (137) has demonstrated that resistance to UVB also increases cold hardness, as does nutrient, drought and other stresses (138). Production of lignin, tannins, suberin, anthocyanins and other secondary compounds occurs in tandem with exposure to environmental stress.

While many induced cross-resistances may be due to cellular modifications, it is more likely that developing leaves (which necessarily lack these modifications) would rely on vacuolar substances to attenuate radiation and modify water relations. Anthocyanins would seem to be good general protectors for a number of reasons:

First, anthocyanins are extremely soluble in water as they occur almost exclusively as glycosides (112) and would therefore readily accumulate in vacuoles. It is important to realize that osmotic stress can be induced by various environmental factors including radiation absorption, temperature extremes and relative water gradients (139), so resistance to these stresses is directly or indirectly dependent upon water relations within tissues.

Secondly, the fact that anthocyanins are glycosylated allows them to bind and transport reactive monosaccharides produced during developmentally or environmentally critical stages. The location of anthocyanins in ray parenchyma of cold-hardy trees (72) might very well serve in this capacity.

Thirdly, anthocyanins have the ability to attenuate UVB if appropriately acetylated with hydroxycinnamic acids. Even without acylation, anthocyanins can significantly attenuate visible radiation, which might be adaptive for young leaf tissues that lack adequate structural protection to avoid photooxidation from high levels of blue light.

I believe that anthocyanins in leaf tissues have a dual function as absorbers of harmful levels and/or wavelengths of radiation and as osmotic adjusters. This second function has at least two environmentally important consequences—when the water potential of the epidermis is lowered, two environmental stresses can be avoided: ice nucleation via freezing events on the leaf surface and drought. Krol et al. (18) speculate that the phenomenon of anthocyanin development in young *Pinus* seedlings may somehow help them establish under a suite of suboptimal environmental conditions including photooxidation, low temperature, water and nutrient stress. Thus, leaf anthocyanins may be triply protective in preventing damage caused, directly or indirectly, by cold temperatures, drought and UV radiation.

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REFERENCES


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