Shared mechanisms of photoprotection in photosynthetic organisms tolerant to desiccation or to low temperature

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A R T I C L E I N F O

A B S T R A C T

Plants that are tolerant of extreme low temperatures, and plants that are tolerant of desiccation, face similar physiological challenges, as low temperatures often result in extracellular ice formation resulting in desiccation of cells. Desiccation due to either low temperatures or water loss leads to severe mechanical as well as oxidative stress, and plants tolerant to either low temperatures or desiccation utilize similar mechanisms to cope with the extreme stress. Key mechanisms to cope with the mechanical stressors associated with cellular crowding include accumulation of both non-reducing sugars as well as LEA proteins. Key mechanisms to cope with oxidative stress include increases in (or the ability to maintain) antioxidant capacity, as well as increased usage of thermal energy dissipation. During both stressors major reductions in photochemical efficiency are observed that correlate with dark retention of the xanthophyll pigments zeaxanthin and antheraxanthin, suggesting sustained forms of thermal energy dissipation persist in these extreme stress scenarios to cope with the increased excitation pressure. The focus of this review is to highlight the shared physiological mechanisms used in both stress scenarios, with a focus on the mechanisms of photoprotection.

1. Introduction to low temperature – and desiccation – tolerances

The relationship between freezing and desiccation stress is well known (Burke et al., 1976; Guy, 1990; Levitt, 1980; Sakai and Larcher, 1987; Sutinen et al., 2001; Xin and Browse, 2000). As a result of the desiccating effects of low temperatures on cells, injury caused by freezing is largely a consequence of cellular dehydration (Sutinen et al., 2001). Therefore, low temperature stress and desiccation stress result in similar physiological effects, and indeed share multiple genes and signalling pathways (Guy et al., 2007; Thomashow, 2010; Krasensky and Jonak, 2012; Gechev et al., 2013). Recent advances in genetics and metabolomics highlight the similarities in the response pathways for these stressors (reviewed in (Krasensky and Jonak, 2012)). Nevertheless, while the effects of both stressors are similar, desiccation tolerant (DT) photosynthetic organisms are not necessarily tolerant of low temperatures, and likewise many low temperature tolerant (LTT) organisms cannot survive desiccating conditions.

Both LTT and DT organisms must deal with strong mechanical and oxidative pressure in the cell. The structural stressors involve (i) destabilization of macromolecular structure due to cellular crowding and subsequent interactions between molecules (Crowe et al., 1992; Vertucci and Farrant, 1995; Hoekstra et al., 2001), (ii) loss of cell compartmentalisation because of membrane fusion and (iii) strong tension between the plasma membrane and cell wall due to cell shrinkage and (for LTT organisms) ice formation (Levitt, 1980; Vertucci and Farrant, 1995; Rascio and La Rocca, 2005; Buchner and Neuner, 2010). In plants undergoing low temperature stress, the requirement for extracellular freezing of water places another mechanical stress on tissues, which must accommodate potentially bulky ice crystal growth during sub-zero temperature conditions (Ball et al., 2004; Roden et al., 2009; Neuner, 2014). The oxidative stressors occur due to stress-induced limitations on photosynthesis and metabolism in general (i.e. molecular mobility (including enzyme activity) is dramatically slowed dawn), while the tissue retains chlorophyll and therefore absorbs light. Such conditions result in a severe imbalance between light absorption and its utilization via carbon metabolism, and necessitate upregulation of photoprotective mechanisms that either dissipate excessive excitation energy or provide protection from damaging reactions.

Recent studies examining the photoprotective strategies used by plants in low temperature conditions (Verhoeven, 2013), and those used by desiccation tolerant organisms in desiccating conditions (Proctor, 2001; Fernández-Marín et al., 2009), have found some interesting similarities in chlorophyll fluorescence and photoprotective pigment responses in the two forms of extreme stress, suggesting that

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there may be some important shared mechanisms of tolerance (García-Plazaola et al., 2012; Verhoeven, 2014). Our goal for this literature review is to provide a comparison of these areas of extreme plant stress tolerance with a focus on photoprotection. We aim to highlight some common mechanisms for tolerance and provide insights into future areas of research investigating these forms of extreme stress tolerance.

1.1. Low temperature tolerant photosynthetic organisms

Photosynthetic organisms vary in their ability to tolerate low temperatures; in fact variation in resistance to winter frost is a major factor in determining species distributions (Bannister & Neuner, 2001; Körner, 2012). The ability to survive extreme low temperature stress (LTT organisms), occurring in species at northern high latitude climates and high elevations, involves a variety of strategies. Freeze tolerance refers to the ability to tolerate ice formation, while freeze avoidance refers to cell types that are able to supercool to temperatures well below 0 °C, primarily via exclusion of heterogeneous ice nucleation within cells (Levitt, 1980; Sutinen et al., 2001; Körner, 2012; Neuner, 2014). Supercooling has been documented in leaves down to temperatures of around −12 °C, and in xylem parenchyma and overwintering buds down to temperatures of −40 °C (Burke et al., 1976; Körner, 2012; Neuner, 2014). Although both strategies have been documented in a variety of photosynthetic organisms, and in some cases within different tissues in a given organism, it seems that the most extreme low temperature tolerant species rely on a strategy of freeze tolerance (Burke et al., 1976; Sutinen et al., 2001; Körner, 2012).

In tolerant organisms, freezing temperatures result in ice formation in the intercellular spaces of tissues. This occurs because the extracellular fluids have a lower solute concentration and therefore higher freezing point than fluids within the cell, and additionally the intercellular spaces contain heterogeneous ice-nucleating agents (Burke et al., 1976). Upon extracellular ice formation, water potential gradients favour ice growth via diffusion of water from plant protoplasts, resulting in shrinkage and desiccation of plant and bryophyte cells (Burke et al., 1976; Buchner & Neuner, 2010; Lenné et al., 2010). The ability of plant cells to withstand desiccation is therefore fundamental to extreme freezing tolerance (Burke et al., 1976; Levitt, 1980; Zwiazek et al., 2001; Körner, 2012).

One of the key differences in the freezing tolerance strategy is whether or not the photosynthetic machinery is retained during the low temperature conditions. Evergreens maintain their leaves or needles during low temperature conditions and thus are able, in principle, to resume photosynthetic activity relatively quickly upon warming. A second group of perennials dismantle most components of the photosynthetic apparatus but maintain the leaves during winter (Lutz, 1996). This is the case of, for instance, *Buxus sempervirens* whose leaves become red in winter due to dramatic changes of their chloroplasts that include the accumulation of protective red carotenoids (Hormaetxe et al., 2004). The same plastids re-differentiate again to photosynthetic chloroplasts in spring (Hormaetxe et al., 2004). Winter-deciduous plants represent a third strategy in which plant leaves senesce in autumn and new leaves must be synthesised in the spring before growth can commence. This review will focus on those LTT plants/organisms that keep their chlorophyll-containing tissues during winter (i.e.: "true evergreens").

In contrast to desiccation tolerance, which can be constitutive, tolerance to low temperatures critically depends upon acclimation. Even the hardiest plants will suffer freeze injury if exposed to low temperatures in a non-acclimated state (Burke et al., 1976; Levitt, 1980). The process of acclimation is induced by shortening photoperiod, but can also be modulated by low temperatures (Levitt, 1980; Bigras et al., 2001).

1.2. Desiccation tolerant photosynthetic organisms

Plants vary considerably in their ability to tolerate desiccating conditions. Extremely tolerant plants, described as "desiccation tolerant" (DT) can survive having their internal water potential equilibrated with the environment, and then upon rehydration are able to resume normal activity (Rascio and La Rocca, 2005). This can mean survival to water potentials as low as −100 MPa (Proctor et al., 2007; Fernández-Marín et al., 2016). Desiccation tolerance is common in seeds and spores. In vegetative tissues it is widespread among terrestrial or intertidal algae, bryophytes and lichens, but is rare in vascular plants, with around 300 species having been documented among ferns and angiosperms (Alpert, 2006). Desiccation tolerance has not been described in photosynthetic tissues of gymnosperms (Alpert, 2006).

Among DT plants, two general strategies have evolved relating to the presence or absence of chlorophyll while in the desiccated state (Fernández-Marín et al., 2016). Plants described as homoiochlorophyllous retain chlorophyll within chloroplasts during desiccation and can thus become photosynthetically active very quickly upon rehydration. This strategy is used by the majority of DT photosynthetic organisms, including all DT-bryophytes and lichens, and most vascular plants. In contrast, poikiochlorophyllous plants degrade chlorophyll and thylakoid membranes upon desiccation, and therefore take longer to recover from a desiccation event (Rascio and La Rocca, 2005). This is a more restricted strategy, so far described in a few species within the monocotyledons only (Fernández-Marín et al., 2016), and will not be included in the scope of this review.

Another important distinction among DT plants occurs between vascular plants and nonvascular plants and algae. Photosynthetic organisms such as bryophytes or lichens encounter desiccating conditions frequently, and are able to desiccate and recover physiological function after rehydration very rapidly. These organisms therefore must maintain constitutive systems for tolerating desiccation and will be designated as DT_c organisms in this review to emphasize the constitutive response. In contrast, most DT vascular plants encounter desiccation conditions less frequently and, due to more complex leaf anatomy, only tolerate desiccation when it occurs more slowly, as the induction of protective processes requires time to be activated. Such plants are therefore termed "modified desiccation tolerant plants" (Rascio and La Rocca, 2005), and will be referred to as DT_i plants in this review to emphasize the induced response. However the distinction between strategies is not always clear, as in some bryophytes desiccation tolerance also relies on inducible mechanisms (Stark, 2017).

2. General mechanisms of desiccation – and low temperature – tolerances: dealing with mechanical stress

2.1. Changes in membrane composition

In conditions of desiccation followed by rehydration, as well as freezing followed by thawing, the primary site of cellular injury is the plasma membrane, thus maintenance of cellular membranes during the desiccation-rehydration cycle is critical to tolerance of desiccation and low temperature conditions (Steponkus, 1984; Crowe et al., 1992;) (Fig. 1). Studies on both DT and LTT plants have demonstrated changes in lipid composition during stress, although in both cases species variation in specific lipid changes have been noted (Sakai and Larcher, 1987; Li et al., 2004; Dinakar and Bartels, 2013; Gasulla et al., 2013; Strimbeck et al., 2015). A decrease in the relative amount of monogalactosyldiacylglyerol (MGDG) via conversion into digalactosyldiacylglyerol (DGDG) and oligogalactolipids, in chloroplast membranes, has been reported both during desiccation in *Craterostigma plantagineum* (Gasulla et al., 2013) and during cold acclimation in Arabidopsis (Moellering et al., 2010). Replacement of cone-shaped MGDG by cylindrical bilayer forming DGDG and oligogalactolipids are proposed to reduce the likelihood of formation of inverted hexagonal II structures