Understanding desiccation tolerance using the resurrection plant *Boea hygrometrica* as a model system

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INTRODUCTION

The productivity and distribution of plants are affected to a large extent by environmental conditions, due to the immutable nature of plants. A major environmental stress experienced by plants occurs during periods of water limitation, i.e., drought. Drought stress differs according to the availability of water and ranges from stochastic periods of mild water deficit to extreme water loss (desiccation). Most plants can withstand drought for a short period, via physiological and morphological changes such as stomatal closure and architecture specialization to reduce water loss and molecular mechanisms underlying DT in the last decade, including specific responses to water stress, such as cell wall folding and pigment-protein complex stabilizing in desiccated leaves. In this review, the insight into the structural, physiological, and biochemical, and molecular alterations that accompany the acquisition of DT in *B. hygrometrica* is described. Finally a future perspective is proposed, with an emphasis on the emerging regulatory roles of retroelements and histone modifications in the acquisition of DT, and the need of establishment of genome sequence database and high throughput techniques to identify novel regulators for fully understanding of the matrix of DT.

Keywords: *Boea hygrometrica*, desiccation tolerance, gene expression regulation, resurrection plant, adaptation

Vegetative tissues of *Boea hygrometrica*, a member of the Gesneriaceae family, can tolerate severe water loss to desiccated state and fully recover upon rehydration. Unlike many other so called “resurrection plants,” the detached leaves of *B. hygrometrica* also possess the same level of capacity for desiccation tolerance (DT) as that of whole plant. *B. hygrometrica* is distributed widely from the tropics to northern temperate regions in East Asia and grows vigorously in areas around limestone rocks, where dehydration occurs frequently, rapidly, and profoundly. The properties of detached *B. hygrometrica* leaves and relative ease of culture have made it a useful system to study the adaptive mechanisms of DT. Extensive studies have been conducted to identify the physiological, cellular, and molecular mechanisms underlying DT in the last decade, including specific responses to water stress, such as cell wall folding and pigment-protein complex stabilizing in desiccated leaves. In this review, the insight into the structural, physiological, and biochemical, and molecular alterations that accompany the acquisition of DT in *B. hygrometrica* is described. Finally a future perspective is proposed, with an emphasis on the emerging regulatory roles of retroelements and histone modifications in the acquisition of DT, and the need of establishment of genome sequence database and high throughput techniques to identify novel regulators for fully understanding of the matrix of DT.

Desiccation tolerance is found commonly in lower plants such as lichens and bryophytes, and are absent in gymnosperms and are found rarely in pteridophytes and angiosperms (Farrant et al., 2007; Porombku, 2011). So far, about 1,300 desiccation-tolerant plants have been described, out of which only 135 species are angiosperms, which are scattered among 13 largely unrelated families (Gaff and Oliver, 2013). Among the dicotyledoneae, the Gesneriaceae contains a variety of resurrection plants. However, DT ability was reported only for several genera including *Boea*, *Ramonda*, *Paraboea*, and *Haberlea* (Müller et al., 1997; Jiang et al., 2007; Huang et al., 2012). In the two families containing the largest number of monocotyledoneae genera of desiccation-tolerant species, the desiccation-tolerant species make up only a small proportion of their genus and these genera only a small fraction of the family (Gaff and Oliver, 2013). This phenomenon suggests that DT has evolved independently from desiccation-sensitive progenitors (Oliver et al., 2000; Gaff and Oliver, 2013). Thus the DT-associated responses and the underlying mechanisms in angiosperm resurrection plants are likely diversified; some are common, while the others are species-dependent.

Gesneriaceae family contains many resurrection species. For example, *Boea hygrometrica* and *Paraboea rufescens*, that are native to the Southeast Asia, and *Haberlea rhodopensis*, *Ramonda myconi*, and *Ramonda serbica*, that distribute mainly in the Balkan Peninsula. This review will focus on the simplified research system of DT in *B. hygrometrica*, structural, physiological, and biochemical,
and molecular alterations that accompany the acquisition of DT in B. hygrometrica. The specificity of the DT of B. hygrometrica will be discussed in comparison with the other resurrection species belonging to the same family that have habitats where drought is only one of the main stresses.

**A SIMPLIFIED MODEL SYSTEM TO STUDY DT USING B. hygrometrica DETACHED LEAVES**

*Boea hygrometrica* is a small, perennial, and herbaceous plant belonging to the Gesneriaceae family. The species is distributed widely from the tropics to northern temperate regions in East Asia and grows vigorously in limestone rocks, where the soil is alkaline and calcium-rich, and dehydration occurs frequently, rapidly, and profoundly. *B. hygrometrica* plants are desiccated and shrink with a withered appearance in dry weather, and become hydrated again after rain in the native habitat (Figure 1). *B. hygrometrica* can be cultivated easily under greenhouse conditions. Seed sets with the aid of manual pollination. The seeds of *B. hygrometrica* are similar in size to *Arabidopsis thaliana* and the number of seeds in one capsule typically exceeds a hundred. The DT ability and ease of handling and maintenance has made *B. hygrometrica* a suitable model system to investigate molecular mechanism of DT.

A remarkable ability of *B. hygrometrica* is that a single detached leaf or leaf disc also possesses the same level of capacity for DT as that of whole plant, which was found only in a sub set of the resurrection plants such as *Craterostigma plantagineum*, *Myrothamnus flabellifolia*, and *Craterostigma nanum* (Gaff and Lovesey, 1984; Bartels et al., 1990; Sherwin, 1995; Jiang et al., 2007). The detached leaves are useful to investigate DT, taking the advantage that these leaves are not affected by interference from developmental regulation and long-distance signaling from other organs during dehydration and rehydration (Jiang et al., 2007). To date, studies using this system have been conducted to characterize the architectural, physiological, cellular, and molecular mechanisms of the DT of *B. hygrometrica*, revealing dehydration responses such as cell wall folding, accumulation of raffinose oligosaccharides, late embryogenesis abundant (LEA) proteins and small heat shock proteins (sHSPs), antioxidative agents, and enzymes and stabilization of photosynthetic protein-pigment complexes (Jiang et al., 2007; Liu et al., 2009; Wang et al., 2009a; Zhang et al., 2013).

*Boea hygrometrica* survives rapid desiccation by air-drying; however, this ability is limited to natural habitats where water is periodically available. *B. hygrometrica* plants grown under well-irrigated conditions in greenhouse conditions are unable to tolerate rapid desiccation, unless pretreated with a dehydration/rehydration cycle, indicating that the slow soil drought and re-irrigation procedure is critical. The non-acclimated and acclimated plants lose water at similar rate although non-acclimated plants fail to revive after rehydration, while acclimated plants recover after rehydration. This characteristic has not been reported for other DT plants, yet the observation that acclimation improves drought, cold and heat tolerance had been reported in many plant species (Bayley et al., 2001; Holmstrup et al., 2002). A common view is that a period of acclimation activates stress-induced gene expression and metabolic changes which in turn are beneficial to plant survival under stress (Ahamed et al., 2012).

**THE BIOLOGICAL CHARACTERS AND STRUCTURAL ADAPTATION OF B. hygrometrica IN RESPONSE TO DEHYDRATION**

**LEAF CURLING AND CELL WALL FOLDING DURING DEHYDRATION**

Adaptive changes in leaf architecture are observed in response to periods of water deficit. These alterations are generally slower responses. For example, dehydration results in leaf shrinkage and curling toward the adaxial surface in many resurrection plants, so that the epidermis hairs on abaxial surface result in a gray-green coloration. The curling of the leaf surface and crowded epidermis hairs on the abaxial surface is considered a protective strategy against photoinhibition and reactive oxygen species.
In agreement, a gene (Jones and McQueen-Mason, 2004; Vicré et al., 2004b; Moore et al., 2008; Wang et al., 2009a). The cell wall remains flexible during dehydration and becomes highly folded, which is helpful to reduce the extent of plasmolysis (Jones and McQueen-Mason, 2004; Vicré et al., 2004b; Moore et al., 2008). By cell wall folding, what is more, damage to the plasma membrane is minimized and the integrity of cell structures and the cell-to-cell communication through plasmodesmata is maintained (Neale et al., 2000; Jones and McQueen-Mason, 2004). The unbalanced folding of cell walls and shrinkage of cells in turn enables leaf curling and reversible folding (Moore et al., 2006, 2008; Farrant et al., 2007). Another process by which the plants can mitigate mechanical stress is by increased vacuolation when the water in vacuoles is replaced by non-aqueous substances (Oliver et al., 2011).

In B. hygrometrica, the contents of cell wall associated proteins and lignin were reduced in desiccated leaves (Wang et al., 2009a; Wu et al., 2009). In agreement, a gene (BGRF1) encoding a cell wall structural glycine-rich protein (GRP), was isolated from the cDNA library of B. hygrometrica leaves dehydrated for 2 h with the help of cDNA microarray approach. GRPs form a large family of heterogenous proteins that contains 60–70% of glycine residues out of the total amount of amino acid residues (Sachetto-Martins et al., 2000). There are two types of GRPs in plants. One contains an RNA-binding domain and is thought to be involved in regulation of RNA processing inside the nucleus or function similarly as that to animal cytoskeleton (Mousavi and Hotta, 2003). The other class of GRPs is thought to be present in the extracellular matrix to form the structural components of plant cell walls (Sachetto-Martins et al., 2000). These GRPs are influenced by external agencies such as water, ozone stress, hormone treatment, wounding, low temperature, etc. It has also been found that water stress results in the induction of several GRP genes in both resurrection and non-resurrection plants (de Oliveira et al., 1996; Neale et al., 2000). Besides, a gene encoding a peptide highly homologous to dirigent proteins and a gene encoding germin-like proteins were identified among the dehydration-responsive genes in B. hygrometrica (Cruz de Carvalho, 2008). Photosynthesis is completely inhibited when water deficiency progresses in many non-resurrection plants, including B. hygrometrica, but in contrast to non-resurrection plants, the process is reestablished soon after rehydration (Farrant and Hussain, 2011; Porembski, 2011; Dinakar et al., 2012). The mechanism underlying photosynthesis changes is not fully understood, however, the following observations have been observed in B. hygrometrica: (1) being homosclorophyllous, it retains chlorophyll contents during desiccation (Deng et al., 2003), (2) chloroplasts assume an irregular shape soon after dehydration, but the thylakoid structure remains visible.
Antioxidant enzymes, osmolytes, and protective macromolecules reduce the metabolic rate (Oliver et al., 2000; Phillips et al., 2002; Martini, 2008). The accumulation of these molecules prevents accumulation of ROS, and accumulate to high levels in dehydrated DT plants. The accumulation in DT plants, including the two European Gesneriaceae species Chirita eberhardtii and Chirita rhodopensis, might be important for establishing the resurrection phenotype in this species (Djilianov et al., 2011). Accordingly, carotenoids and LEA proteins are implicated to play a role in this process.

THE ACCUMULATION OF PROTECTIVE MOLECULES IN RESPONSE TO DEHYDRATION

Antioxidant enzymes, osmolytes, and protective macromolecules accumulate at high levels in dehydrated DT plants. The accumulation of these molecules prevents accumulation of ROS, and protects membranes and proteins by forming a glass state, which reduces the metabolic rate (Oliver et al., 2000; Phillips et al., 2002; Bartels and Hussain, 2011).

Sugars

One of the principal osmolytes that accumulates during desiccation is sucrose. Sucrose acts as an osmoprotectant to stabilize the structure of macromolecules to protect biological membranes (Martinelli, 2008), and may function as a signaling component to regulate carbohydrate status, growth, and energy metabolism. Besides, oligosaccharides such as raffinose and trehalose accumulate in many species. Trehalose may also prevent crystallization of sucrose during drying (Müller androssowii et al., 1996; Babu et al., 2004; Park et al., 2005). LEA proteins were first discovered during the final stages of seed development (Dure and Chalan, 1981). Further studies have shown that LEA proteins accumulate in response to drought, freezing, salt stress, and by treatment with the phytohormone ABA (Shao et al., 2005; Tunnacliffe and Wise, 2007). Studies also revealed that LEA proteins are produced in vegetative tissues and seeds both in desiccation-sensitive and tolerant plants during drought (Pathkowsk et al., 1990; Battaglia et al., 2006; Hundertmark and Hincha, 2008). Increased drought tolerance has been found in transgenic plants such as barley, Tamarix androsaemum and Brassica napus which overexpress LEA genes (Xu et al., 1996; Babu et al., 2004; Park et al., 2005). These observations imply that the accumulation of galactinol and raffinose may contribute to the osmotic protection in fully desiccated leaves in at least the above-mentioned resurrection species. Hereby, the function of galactinol in DT will need further investigation.

In addition, raffinose also accumulates soon after dehydration in B. hygrometrica. To the contrary, raffinose (and sucrose) accumulated only when leaf RWC decreases to 25% or lower in H. rhodopensis (Djilianov et al., 2011). It was proposed that the initial high sucrose and raffinose concentration in H. rhodopensis, as revealed by the comparison with its non-DT relative Chirita eberhardtii, might be important for establishing the resurrection phenotype in this species (Djilianov et al., 2011). Therefore it appears that although the dynamics of raffinose accumulation may vary in individual species, the high level of raffinose in the early stage of dehydration is common to B. hygrometrica and H. rhodopensis, and the maintaining constant high levels of sucrose and raffinose might be a specific adaptation to be able to survive a very rapid dehydration in these species.

Protective proteins

In B. hygrometrica it was inferred that mechanisms exist which prevent protein aggregation and degradation (Jiang et al., 2007). Two major classes of protective proteins are sHSPs and LEA proteins, which constitute the largest group of hydrophilins in plants (Ingram and Bartels, 1996; Battaglia et al., 2005). The hydrophilins are predicted to protect proteins and macromolecules from dehydration by creating a water hydration “shell” (Oliver et al., 2011). LEA proteins were first discovered during the final stages of seed development (Dure and Chalan, 1981). Further studies have shown that LEA proteins accumulate in response to drought, freezing, salt stress, and by treatment with the phytohormone ABA (Shao et al., 2005; Tunnacliffe and Wise, 2007). Studies also revealed that LEA proteins are produced in vegetative tissues and seeds both in desiccation-sensitive and tolerant plants during drought (Pathkowsk et al., 1990; Battaglia et al., 2006; Hundertmark and Hincha, 2008). Increased drought tolerance has been found in transgenic plants such as barley, Tamarix androsaemum and Brassica napus which overexpress LEA genes (Xu et al., 1996; Babu et al., 2004; Park et al., 2005). LEA proteins help to minimize damages caused due to stress by functioning in the protection of membranes and proteins, and alleviate the increase in ion concentration (Ingram and Bartels, 1996; Shao et al., 2005; Tunnacliffe and Wise, 2007). In the desiccated state, LEA proteins along with sugars form a “glassy” state (Butink and Leprince, 2004). Based on amino acid sequence homology and specific structural features LEA proteins were classified into five groups (Dure et al., 1989). So far, only two genes encoding group 4 LEA proteins had been cloned from B. hygrometrica. Over-expression of both BhLEA1 and BhLEA2 improved transgenic tobacco drought tolerance as evidenced by increased photosynthetic efficiency and membrane integrity, increased abundance of...
ROS scavenging enzymes such as superoxide dismutase (SOD) and peroxidase (POD; Liu et al., 2009). Furthermore, chloroplastic membranes-bound proteins such as PBO and LHChI were highly stable in drought-stressed *B. hygrometrica* transgenic plants, and chloroplastic stroma proteins RbcL were better conserved in drought-stressed *B. hygrometrica* transgenic plants, highlighting the important roles of LEA proteins in the protection of photosynthetic proteins (Liu et al., 2009).

Similarly, sHSPs protect proteins from both aggregation and dehydroxylation by acting as molecular chaperones (Garcia et al., 2012). Ten genes encoding hSPs were cloned from *B. hygrometrica*, among which, six cytosol-targeted hSPS coding genes were induced after desiccation and tended to remain highly abundant during rehydration (Zhang et al., 2013). It has been established that stress conditions affect cellular environment at least in part by disturbing protein folding. There are two processes to eliminate unfolded and misfolded proteins in the cells, one in the endoplasmic reticulum associated degradation (ERAD) and the other cytoplasm protein response (CPR; Mishiba et al., 2015). The finding of dehydroxylation-inducible cytosol sHSP coding genes indicated a role of sHSPs in the stabilization of cytosolic proteins.

**Antioxidants and ROS scavenging enzymes**

A large number of stressful conditions such as salinity, drought, highlight, toxicity, pathogens cause extra ROS (Müller, 2012). The complex antioxidant defense system of plants consists of non-enzymatic and enzymatic components. Recent studies indicate that these components exist in different organelles such as chloroplasts, mitochondria, and peroxisomes (Pang and Wang, 2008). Non-enzymatic components include the major cellular redox buffers ascorbate (AAs) and glutathione (GSH) as well as tocopherol (vitamin E), carotenoids, and phenolic compounds (Müller, 2012). Interacting with numerous cellular components, these antioxidants modulate processes from mitosis and cell elongation to senescence and cell death (De Pinto and De Gara, 2004). (Poly)-phenols together with flavonoids appear to be particularly important in resurrection plants acting as “sun screen” pigments to shade the desiccated photosynthetic apparatus, and which will help to avoid \( \text{O}_2 \) formation (Farrell et al., 2003; Kraner and Birtic, 2005). In *R. serbica*, the content of phenolic acids is found to be unusually large in comparison with other plants (Booker and Miller, 1998; Sgherri et al., 2004). The enzymatic components comprise of several antioxidant enzymes such as SOD, catalase (CAT), guaiacol peroxidase (GPx), ascorbate peroxidase (APX), monodehydro ascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR; Nistor and Foyer, 1998). These enzymes operate in different subcellular compartments and respond in concert when cells are exposed to oxidative stress. In *Craterostigma wilmsii* and *X. viscosa*, vegetative tissues show increased expression of enzymatic antioxidants genes such as APX, GR, and SOD during drying or rehydration (Ingram and Bartels, 1996; Sherwin and Farrar, 1998). Mowla et al. (2002) identified a novel stress-inducible antioxidant enzyme *XvPer1* from the resurrection plant *X. viscosa*, which may function to protect nucleic acids within the nucleus against oxidative injury. In *B. hygrometrica*, a protein annotated to polyphenol oxidase precursor was identified to be induced by dehydration, along with the proteins annotated to glutathione peroxidase and glutathione S-transferase (Jiang et al., 2007). In consistence, the content of antioxidants GSH and the activity of ROS scavenging enzyme polyphenol oxidase are increased in *B. hygrometrica* during desiccation (Jiang et al., 2007). Polyphenol activity was also found higher in desiccated leaves in *R. serbica* and *H. rhodopensis* (Jovanovic et al., 2011). In a recent study it was found out that polyphenols protect chloroplast membranes during plant desiccation and recovery by helping the membrane to mitigate oxidation damage and facilitate in starting the photosynthesis when the plant rehydrates (Georgieva et al., 2010).

**EVALUATION OF THE MOLECULAR MECHANISMS OF DT IN *B. hygrometrica***

The molecular mechanisms of DT in resurrection plants have been investigated since the 1990s (Bartels et al., 1998). Numerous dehydration-inducible genes have been cloned from resurrection plants as summarized by a series of reviews (Ingram and Bartels, 1996; Farrant et al., 2007; Farrant and Moore, 2011; Ghez et al., 2012; Gaff and Oliver, 2013). The advances of “omics” technologies have greatly facilitate the discovery of DT-related genes and the understanding of the molecular mechanisms of DT. Since the last century, technologies of miRNA differential display, proteomic, macroarray hybridization have been applied to study the DT mechanism in *B. hygrometrica*, which led to the discovery of many dehydration-induced genes and proteins (Deng et al., 1999; Jiang et al., 2007; Wang et al., 2009a).

Molecular studies based on these finding have revealed a general regulation module that transcription factors control the dehydration-induced expression of functional genes downstream of phytohormone-dependent and -independent signal transduction. Plants respond immediately to water stress by various kinds of physiological responses including a rapid increase in ABA concentration. Both ABA-dependent and -independent signal pathways have been revealed in the activation of gene expression. The genes encoding the protective proteins such as aldehyde dehydrogenase, heat shock factors and LEA proteins are thought to be regulated by ABA-directed signal pathways (Kirsch et al., 2001; Deng et al., 2006; Wu et al., 2011). Dehydration-triggered gene expression is regulated by a cascade of signaling molecules such as transcription factors, calmodulins, and kinases and phosphatases. One example of hormonal signaling in *B. hygrometrica* is ABA-dependent synthesis of galactinol and raffinose family oligosaccharides (RFOS). Both BtGoLS1 and BRF8S were induced by ABA (Wang et al., 2009b; Wang et al., 2012). The activation of BtGoLS1 was achieved by the regulation of a dehydration and ABA-inducible WRKY transcription factor which binds to the **W**-box elements in the promoter region of BtGoLS1 (Wang et al., 2009b).

The role of other phytohormones in DT regulation in *B. hygrometrica* has not be investigated so far, however, the study in *H. rhodopensis*, another DT species in Gennericiaceae, had revealed the active participation of jasmonic acid, salicylic acid, cytokinins, and auxins in the dehydration response (Dulaman et al., 2013).

As proposed by the authors, DT appears to be strongly influenced by the earliest and very high accumulation of JA and ABA,
which coincides with the accumulation of early up-regulated transcripts, and the steady high levels of SA during the whole process of desiccation (Georgieva et al., 2012; Dhilnav et al., 2013). A forthcoming study on the hormone changes during dehydration and rehydration in *B. hygrometrica* will shed light on the specificity and universality in the hormone regulation of DT molecular events among species in Gesneriaceae.

Abscisic acid and calcium have been shown to interact in regulation of dehydration-induced gene expression in *B. hygrometrica*. Calcium regulates expression of a dehydration-inducible gene *BhC2DP1* in *B. hygrometrica* (Zhang et al., 2012). *BhC2DP1* encodes a small protein with a single C2 domain protein, which is capable of binding Ca$^{2+}$. Constitutive expression of *BhC2DP1* in Arabidopsis resulted in an ABA-hypersensitive phenotype, which could be rescued by supplementing Ca$^{2+}$-chelating agent EGTA to growth media. Thus we propose that Ca$^{2+}$ is necessary for the function of *BhC2DP1* in response to ABA. Consistent with this hypothesis *BhC2DP1* transcripts accumulate soon after dehydration and exposure to exogenous Ca$^{2+}$. *BhC2DP1* transcription was suppressed by ABA and EGTA, however, was promoted when ABA and EGTA were simultaneously applied. This observation suggests that the transcriptional regulation of *BhC2DP1* by ABA is Ca$^{2+}$ dependent. Fine-tuning of *BhC2DP1* expression in response to drought highlights the role of exogenous calcium in the DT response.

Calcium is an essential plant macronutrient with key structural and signaling roles and it is rich in the limestone-based alka-line soil (Ji et al., 2009). Excessive Ca$^{2+}$ in the rhizosphere may also cause soil alkalization and Ca$^{2+}$ toxicity by preventing the germination of seeds, reducing plant growth rate and formation of tiny yellowish or gold spots in the cell walls of fruits (White and Bradly, 2003; Song et al., 2011). Interestingly, many species in Gesneriaceae family favor calcareous massifs, including both the desiccation-tolerant *B. hygrometrica*, *R. myconi*, *R. serbica*, *P. rufescens*, *H. rhodopensis*, and the desiccation intolerant species of *Chirita* spp. and *Monopophyllia* spp. (Picó and Riba, 2002; Sgherri et al., 2004; Georgieva et al., 2008; Kiew, 2009; Huang et al., 2012).

The high occurrence of DT species and calciphiles in Gesneriaceae suggests a possible link between environmental calcium and DT. However, does calcium indeed involve in the regulation of DT mechanisms, how these plants limit high calcium damage, and how they balance the calcium signal from high calcium stress and dehydration stress, and if the high calcium environment benefits the evolution of DT in the resurrection species of Gesneriaceae are open to question.

Likewise, dehydration of resurrection plants in their natural habitats frequently occurs in combination of different abiotic stresses, each with the potential to exacerbate the damage caused by the others. This is particularly true in the case of Gesneriaceae resurrection plants that have habitats where drought is not the only main problem. For example, *H. rhodopensis* plants grow in shady rock crevices on limestone at altitudes of 100–1,700 m in the central to north part in Balkan Mountains and the South in Rhodope Mountains, Ramonda serbica inhabits the shallow organo-mineral soil (pH 7.7) that develops in crevices on northern-facing carbonate rocks in the gorges in the Balkan Peninsula, and *B. hygrometrica* is native of vast area from Northern China to Southeast Asia (Jiang et al., 2007; Rakić et al., 2009; Daskalova et al., 2011; Petrova et al., 2013), growing also on limestone at altitudes of 200–1,320 m. In these places, the high temperature and high irradiance in summer will increase the rate of water loss and the low temperature beneath 0°C in winter could cause freezing. It has been revealed that the effects of dehydration on photochemical activity of PSII and PSI and photosynthetic oxygen evolution was stronger when desiccation was carried out at high temperature (38°C) or becomes irreversible damaged during desiccation at high light intensities (350 μmol m$^{-2}$ s$^{-1}$) (Georgieva et al., 2008, 2010; Mihalova et al., 2011). However, at least the populations in the northern temperate zone are able to tolerate several cycles of desiccation and rehydration under high temperature and intensive irradiation conditions in summer, and to survive the freezing temperature in winter after gradually dehydrated to an “anhydrobiosis” (quiescent and desicated) stage during autumn. The similarity of the habitats of these Gesneriaceae resurrection plants highlights the specificity of the DT mechanisms of these plants. Studies on genetic model plants have shown that there are multiple stress perception and signaling pathways, some of which are specific, but others may cross-talk at various steps. It has been established that cold acclimation increases plant freezing tolerance via CBF regulatory hub and over-expressing CBF3/DEBF1a, and consequently the CBF regulon, are not only more freezing tolerant than control plants, but are also more tolerant of dehydration stress caused by either drought or high salinity (Kasuga et al., 1999; Thomashow, 2010). Influx of calcium is an important second messenger involved in activating the cold acclimation response (Duehrty et al., 2009). Whether low temperature and rhizosphere calcium and alkalization have an impact on the regulation of DT mechanisms in *B. hygrometrica* and others will help to elucidate the scientific mechanisms behind the adaptive evolution and the DT acquisition of resurrection plants in Gesneriaceae.

### CONCLUSIONS AND FUTURE PERSPECTIVES

As reviewed above, the characterization of *B. hygrometrica* demonstrates that a number of effective, protective mechanisms are induced upon dehydration. A growing body of evidence has suggested that the adaptation of resurrection plants to dry environments is due to novel regulation of existing genes. Changes in gene expression result in morphological and physiological adaptations which enable survival in a desiccated state. In addition to the general regulation module of dehydration-induced gene expression on transcriptional level, there are two novel aspects that probably worth of noticing for DT-associated genetic regulation.

One of the newly recognized regulators in DT is retroelement. The role of retroelement in DT has been illustrated in the case of *C. plantagineum*. A series of studies have shown that the dehydration-related ABA-inducible retroelement gene *CDT-1* could direct the synthesis of a double-stranded 21 bp short interfering RNA (siRNA), which opened the metabolic pathway for DT through activation of stress-responsive genes (Furini et al., 1997; Smith-Espinosa et al., 2005; Hilbricht et al., 2008). As a major type of transposons, retroelements may silence or alter expression of genes adjacent to insertion sites and generate newly acquired exons (exapted) via transposition,
contribute to chromosomal rearrangements via recombination, epigenetically alter regional methylation patterns, and provide template sequences for RNA interference (Boyko and Kovarichuk, 2011; Moroz and Pazsakowska, 2011; Guizat and Schmid, 2012). The transcriptional activation from the transposons may also trigger locus-specific siRNA mediated DNA-directed DNA methylation (Hilbert et al., 2008; Rigal and Mathieu, 2011; Zhang and Zha, 2011). Transposon elements can rapidly differentiate genomes within and between species has been illustrated (Piegu et al., 2006; Venner et al., 2009; He et al., 2012). A biological diversity investigation on the patterns of genetic structure of *B. mycormi* populations in eight mountain regions has revealed high genetic differentiation between geographical regions (20%) and among populations within regions (9%), (Dubucq et al., 2008). To investigate the DT-associated retrotransposon elements from *B. hygrometrica* and other resurrection species will bring interesting insights into the evolution of Genusaeaceae, species differentiation, and the acquisition and regulation of DT ability in this family.

The other type of the possible novel regulators in DT may be represented in chromatin modification. Particularly, recent studies have linked histone modification with drought tolerance (Bruce et al., 2007; Kim et al., 2010). There are at least eight distinct types of modifications found on histones including the well-informed acetylation, methylation, and phosphorylation (Kouzarides, 2007). The increase of H3K4 trimethylation and H3K9 acetylation in *Arabidopsis* is associated with drought induced expression of stress response genes (Chinhunyu and Zha, 2009). Recent research shows that H3K4me3 modification mediate the rapid induction of trainable genes in the second round of dehydration, which is a sign of stress memory in plants (Ding et al., 2012). Besides, histone modification may also display a transient up or down regulation during stress response that can also affect target gene expression (Soslol et al., 2007). Transient chromatin modifications mediate acclimation response and heritable chromatin modifications provide within-generation and trans-generational stress memory (Kouzarides, 2007). Because *B. hygrometrica* has to undergo a drought acclimation before it gains the DT ability, it may be similar with *Arabidopsis* which keeps a drought memory that brings it a more effective desiccation response. It is possible that the level of a single type of histone modification or even a combined pattern is altered and kept in *B. hygrometrica* during drought acclimation, which results in the activation of a cascade of downstream genes. Further study on the transient or long term chromatin modifications that regulate gene expression for acquisition of DT will expand the regulation frame of gene expression in resurrection plants.

Definitively, mechanisms of DT could be dissected further with the availability of a genetic system that enables gain and loss of function experiments. Currently the application of genetic approaches is limited by the inability to transform *B. hygrometrica* and the lack of genome sequence information of this species. Zhang et al. (2011) have analyzed in a systematic way representation chloroplast and mitochondrial genomes of *B. hygrometrica* and the results provide information for a better understanding of organellar genome evolution and function. Nuclear genome sequencing of *B. hygrometrica* is undergoing. Meanwhile, transformations of large genomic DNA fragments from resurrection plant as the donor to *Arabidopsis* via transformation-competent binary bacterial artificial chromosome (BIBAC) vectors have been employed as a genetic tool for genome-wide screening of functional genes, gene clusters, quantitative trait loci (QTL), transposon elements, chromatin modifications, and other genomic elements. These efforts will help to extend our understanding on the mechanisms of DT acquisition and subsequently facilitate the improvement of drought tolerance of crops and other plants with economic or ecological importance, which is highly desired in the background of global warming.

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**REFERENCES**

Ahamed, A., Murai-Hatano, M., Ishikawa-Sakurai, J., Hayashi, H., Kawamura, Y., and Uemura, M. (2012). Cold stress-induced acclimation in rice is mediated by root-specific aquaporins. *Plant Cell Physiol.* 53, 1445–1456. doi: 10.1093/pcp/pct099

Albani, F. M., Mandel, F. V., Ferrari, M., Cantoni, B., Puliga, S., et al. (1999). Galectin in the leaves of the resurrection plant *Boea hygrometrica*. *Physiologia Plantarum* 104, 499–505. doi: 10.1111/j.1399-3054.1999.00120.x

Allen, R. C., Zhang, J., Bai, A., Ho, T-H. D., Wu, R., and Nguyen, H. T. (2004). HVIAD, a LEA gene from barley confers dehicles resistance in transgenic rice (Oryza sativa L.) via cell membrane protection. *Plant Sci.* 166, 857–862. doi: 10.1016/j.plantsci.2003.11.023

Bartels, D., and Havaux, M. S. (2011). "Resurrection plants: physiology and molecular biology," in *Plant Drought Tolerance*, eds U. Lutting, B. Eck, and D. Bartels (Berlin: Springer), 359–384.

Bartels, D., Schoo, K., Freitag, G., Patkowski, D., and Salihamidzic, F. (1999). Molecular cloning of obscure acid-modulated genes which are induced during drought acclimation of the resurrection plant *Cassonigrella platensis*. *Planta* 181, 27–34. doi: 10.1007/BF00212211

Bayley, M., Petersen, S. O., Knigge, T., Köhler, H., and Holmstrup, M. (2001). Drought acclimation confers cold tolerance in the soil coelomate *Folsomia candida*. *J. Insect Physiol.* 47, 1197–1204. doi: 10.1016/S0022-1910(01)00731-4

Brooker, F., and Miller, J. E. (1998). Phenylpropanoid metabolism and phenolic composition of soybean ( Glycine max L.) leaves following exposure to ozone. *J. Exp. Bot.* 49, 1191–1202. doi: 10.1093/jxb/49.324.1191

Bryksin, A., and Kovarichuk, I. (2011). Genome instability and epigenetic modifications: heritable responses to environmental stress? *Curr. Opin. Plant Biol.* 14, 260–266. doi: 10.1016/j.pbi.2011.03.005

Butinik, J., and Lapinscik, O. (2004). Glass formation in plant anhydrobiotes: survival in the dry state. *Cytobios* 124, 215–228. doi: 10.1016/j.cytobio.2006.02.011

Bruce, J. A. T.; Mathes, C. M., Napier, A. J., and Pickett, A. J. (2007). Stressful "memories" of plant evidence and possible mechanisms. *Plant Sci.* 173, 603–618. doi: 10.1016/j.plantsci.2007.06.002

Chinnusamy, V., and Zhu, J. (2009). Epigenetic regulation of stress responses in plants. *Curr. Opin. Plant Biol.* 12, 15. doi: 10.1016/j.pbi.2008.12.008

Crus de Carvalho, M. H. (2008). Drought stress and reactive oxygen species: production, scavenging and signaling. *Plant Signal. Behav.* 3, 170–175. doi: 10.4161/psb.3.3.5336

"fpls-04-00446" — 2013/11/9 — 19:37 — page 7 — #7
Dankov, E., Domtchev, S., Yabun, G., Minkov, I., and Taneva, V. (2011). A strategy for conservation and investigation of the protected resurrection plant Haberlea rhodopensis. Tr. Inst. Bot. 61, 41–60. doi: 10.3981/trinbot.6.1568

Dong, X., Hu, Z., and Wang, H. (1999). mRNA differential-display visualized by silver staining tested on gene expression in resurrection plant Boa hygrometrica. Plant Mol. Biol. Rep. 17, 1–7. doi: 10.1007/BF02735167

Dong, X., Hu, Z., and Wang, H. (2000). A homoeodomain zipper gene from Craterostigma plantagineum regulates abscisic acid-responsive gene expression and physiological responses. Plant Mol. Biol. 41, 469–489. doi: 10.1023/A:10062253-00000

Djilianov, D., Dobrev, P., Moyankova, D., Vankova, R., Georgieva, D., Gajdošová, S., Georgieva, T., Christov, N., and Djilianov, D. (2012). Identification of desiccation-regulated genes by cDNA-AFLP in Haberlea rhodopensis: a resurrection plant. Aust. J. Plant Physiol. 39, 568–573. doi: 10.1071/PP12308

Dutta, Al., De, S., and Roy, K. (2011). A model for the resurrection plant, Craterostigma plantagineum: a resurrection plant. Ann. Bot. 107, 117–126. doi: 10.1093/aob/mcr274

Dvornic, C., Dillmann, D., and Bartels, D. (2012). Photosynthesis in desiccation tolerant plants: energy metabolism and antioxidative stress defense. Plant Sci. 182, 29–41. doi: 10.1016/j.plantsci.2011.01.018

Farrant, J. M., and Moore, J. P. (2011). Programming desiccation-tolerance: from resurrection of selected angiosperm resurrection plants. Nature 475, 787–795. doi: 10.1038/nature10203

Farrant, J., Brandt, W., and Lidsey, G. G. (2007). An overview of mechanisms of ‘train’ transcriptional responses in resurrection plants. J. Exp. Bot. 58, 2799–2809. doi: 10.1093/jxb/erm253

Farrant, J. M., Vanden Willigen, C., Loffell, D. A., Bartsch, S., and Whitney, T. E. (2005). A comparison of phytochrome apparatus of the desiccated leaves of the resurrection plant Boa hygrometrica with its non-tolerant relative Christia heterosepalum in response to dehydration and rehydration. Plant Sci. 165, 851–861. doi: 10.1016/j.plantsci.2004.10.002

Farrant, J. M., and Whiteway, E. (2011). Photosynthesis in desiccation tolerant plants: energy metabolism and antioxidative stress defense. Plant Sci. 182, 29–41. doi: 10.1016/j.plantsci.2011.01.018

Furini, A., Koncz, C., Salamini, F., and Bartels, D. (1997). High level transcription of stress-related genes in Arabidopsis thaliana following dehydration. Planta 204, 477–486. doi: 10.1007/s00425-006-0580-7

Georgieva, R., Lensk, S., and Bruchmann, C. (2008). Responses of the resurrection plant Haberlea rhodopensis to high irradiance. Photosynthetica 46, 206–215. doi: 10.1007/s11099-008-9014-8

Georgieva, K., Border, A., and Burchell, C. (2009). Chromosome rearrangements in resurrection plant Boa hygrometrica. Cytogenet. Genome Res. 127, 50–64. doi: 10.1159/000174035

Holmstrup, M., Hedlund, K., and Boriss, H. (2002). Drought acclimation and acute desiccation stress. Plant Physiol. 128, 473–480. doi: 10.1104/pp.004253

Ingram, J., and Bartels, D. (1996). The molecular basis of dehydration tolerance leading to resurrection of the plant Craterostigma plantagineum. New Phytol. 137, 887–897. doi: 10.1046/j.1469-8137.2000.00240.x

Ingram, J., and Bartels, D. (1996). The molecular basis of dehydration tolerance leading to resurrection of the plant Craterostigma plantagineum. New Phytol. 137, 887–897. doi: 10.1046/j.1469-8137.2000.00240.x

Jones, R. D. (2009). A resurrection plant. New Phytol. 183, 41–60. doi: 10.1111/j.1469-8137.2009.03012.x

Jurkat, A. (2003). An investigation into the role of light during desiccation of plant cells. Ph.D. thesis. University of Aberdeen.

Kasuga, M., Liu, Q., Yamaguchi-Shinozaki, S. M. K., and Shinozaki, K. (1999). The ABF family of proteins regulates abscisic acid-responsive gene expression and physiological responses. Plant J. 19, 349–359. doi: 10.1046/j.1365-313X.1999.00785.x

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Arabidopsis thaliana: a model system for plant molecular biology. Annu. Rev. Genet. 35, 559–608. doi: 10.1146/annurev.genet.35.112699.235539

Kowalczyk, A., and Jones, D. (2001). Ar...
Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. 

Moore, J. P., Farrant, J. M., and Driouich, A. (2008). A role for pectin-associated proteins: a family or just proteins with a common motif? 

Mishiba, K., Nagashima, Y., Suzuki, E., Hayashi, N., Ogata, Y., Shimada, M., Muller, J., Sprenger, N., Bortlik, K., Boller, T., and Wiemken, A. (1997). Desiccation response of the leaf cell wall to desiccation in the resurrection plant Craterostigma plantagineum encodes a plastid-targeted protein with DNA-binding activity. 

Müller, J., Sprenger, N., Bortlik, K., Boller, T., and Wiemken, A. (1997). Desiccation tolerance in resurrection plants. 

Mirouze, M., and Paszkowski, J. (2011). Epigenetic contribution to stress adaptation in plants. 

Mitra et al. DT in plants. 

Kouzarides, T. (2007). Chromatin modifications and their function. 

Kranner, I., and Birtic, S. (2005). A modulating role for antioxidants in desiccation response. 

Kouzarides, T. (2007). Chromatin modifications and their function. 

Kranner, I., and Birtic, S. (2005). A modulating role for antioxidants in desiccation response. 

Kumar, R., Kaur, R., and Tuteja, N. (2009). Reactive oxygen species (ROS) contribute to water deficit-induced stomatal closure in rice (Oryza sativa). 

Kranner, I., and Birtic, S. (2005). A modulating role for antioxidants in desiccation response. 

Kouzarides, T. (2007). Chromatin modifications and their function. 

Kumar, R., Kaur, R., and Tuteja, N. (2009). Reactive oxygen species (ROS) contribute to water deficit-induced stomatal closure in rice (Oryza sativa). 

Kumar, R., Kaur, R., and Tuteja, N. (2009). Reactive oxygen species (ROS) contribute to water deficit-induced stomatal closure in rice (Oryza sativa).
Vicré, M., Lerouxel, O., Farrant, J., Lerouge, P., and Driouich, A. (2004b). Insights into the cellular mechanisms of desiccation tolerance among angiosperm resurrection plant species. Plant Cell Environ. 27, 1365–1380. doi: 10.1111/j.1365-3040.2004.01212.x

Vicré, M., Farrant, J. M., and Driouich, A. (2004a). Differential expression of sHSP gene family members from the resurrection plant Boea hygrometrica. Physiol. Plant. 120, 229–238. doi: 10.1111/j.0031-9317.2004.02234.x

Wu, H., Shen, Y., Hu, Y., Tan, S., and Lin, Z. (2011). A phytohormone signaling pathway mediates drought-induced osmotic tolerance in transgenic tobacco. J. Plant Physiol. 168, 935–943. doi: 10.1016/j.jplph.2010.09.019

Xu, D. P., Du, X. L., Wang, B. Y., Hong, B. M., Hu, T. H., and Wu, B. (1996). Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol. 110, 249–257.

Zhang, H. M., and Zhu, J. K. (2011). RNA-directed DNA methylation. Curr. Opin. Plant Biol. 14, 142–147. doi: 10.1016/j.pbi.2011.02.005

Zhang, L., Si, F., Wang, L., Qi, D., Zhu, Y., and Dong, X. (2012). A small C2-domain protein from the resurrection plant Boea hygrometrica promotes plant responses to abiotic stress. J. Exp. Bot. 63, 11–27. doi: 10.1073/pnas.1104905108

Zhang, T., Fang, Y., Wang, X., Dong, X., Zhang, X., Hu, S., et al. (2011). The complete chloroplast and mitochondrial genome sequences of Boea hygrometrica insights into the evolution of plant organellar genomes. PLoS ONE 7:e30531. doi: 10.1371/journal.pone.0030531

Zhang, Z., Wang, B., Sun, S., and Dong, X. (2013). Molecular domino and differential expression of shHSP gene family members from the resurrection plant Boea hygrometrica in response to abiotic stresses. Biologia 68, 651–661. doi: 10.2478/s11756-013-0204-4

Zhu, Y., Wang, Z., Jing, Y. J., Wang, L. L., Liu, X., Lin, Y. X., et al. (2009). Ecopic over-expression of BbHsf1, a heat shock factor from the resurrection plant Boea hygrometrica, leads to increased thermotolerance and retarded growth in transgenic Arabidopsis and tobacco. Plant Mol. Biol. 71, 451–467. doi: 10.1007/s00425-007-0612-1

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