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Physiological Response of the Resurrection Fern Subjected to Environmental Stress as Shown by Plant Cell Membrane Integrity

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Abstract

Resurrection fern has a unique ability to maintain cell wall integrity when the plant cell is desiccated. It uses proteins such as late embryogenesis proteins and heat shock proteins to maintain their cellular functions. The purpose of this experiment is to determine the effects of environmental stressors on the physiological response of the resurrection fern (*Pleopeltis polypodioides*). The physiological response of resurrection fern plants was subjected to various temperatures (−50˚C, 0˚C, 25˚C, and 50˚C) for 7 days. Results indicated that there was a significant difference between hydrated and desiccated ferns based on the temperature. Additionally, electrolyte leakage measurements confirmed cell damage following exposure to temperature extremes of −50˚C and 50˚C.

Keywords

Adaptive Mechanisms, Plant Stress, Plant Cell Wall, Cell Leakage

1. Introduction

This experiment will help better the understanding of environmental stressors, how plants are affected by external factors, and adapted mechanisms in plants. For example, a plant's adaptive mechanisms can help explain why and how crops thrive in extreme climates. If these mechanisms are understood, scientists could transpose those genes capable of surviving desiccation into food crops where drought will not negatively impact their growth. This information could also help with problems associated with climate change. Carbon dioxide, the gas that causes the greenhouse effect, is starting to spike, causing global warming and climate change [1]. As the climate warms, plants will need to be more tolerant of these changes so that they can continue to be sources of food for life. If these plants become more tolerant to environmental stressors, this will allow for the opportunity to grow food-producing crops in otherwise barren areas. The ability to engineer more tolerant plants would also be a breakthrough in space travel and the colonization of planets because they could theoretically survive in the harshest conditions, such as a limited water supply or extreme temperatures [2]. This experiment aims to determine the effects of environmental stressors, specifically heat stress on the physiological response of the resurrection fern (*Pleopeltis polypodioides*). The heat stressors affected the physiological response of the resurrection fern.

The resurrection fern is a neotropical epiphytic fern, meaning that it is native to central and South America. The fern's resilience gives it an extensive geographic range [3]. The resurrection fern is commonly found on oaks, magnolias, and elm trees. It needs a minimal amount of water, sunlight, and soil and does not use anything from its host [4]. This plant is considered highly unusual because of its ability to lose 97% of its water, while most plants die after losing only 15% of their water content. When dehydrated, the fern curls into a cylindrical shell [5]. The dehydrated fern exposes the scaly dorsal surface on the underside of the frond to protect the photosynthetic organelles of the frond from the sun. The fern's vacuole solidifies during the desiccation process. The fern only unfurls when water returns. Scientists agree that the resurrection fern could live in this dehydrated state for at least 100 years. This desiccated state is similar to a dormant state like hibernation where the fern unfurls by quickly forcing the new water directly onto the fronds [3].

Ferns are gymnosperms, non-flowering plants that reproduce using spores. They are usually made of one or more fronds attached with a stalk to a rhizome, a specialized root-like stem. Fronds are composed of smaller leaves attached to the stalk in the shape of a sword or blade. The fronds are made of four parts: the apex, or the tip of the frond, the midrib or the stem in the center, the pinna or the single leaflets connected to the midrib, and sometimes pinnules or the little segments that make up the pinna [6]. The resurrection fern is part of a loose group called fern-allies. This group is distinct in how their spores are made at the base of their leaves or use special branches to hold them [7].

This species is one of the few of its kind that survived the Carboniferous era. There are many possible reasons why the resurrection fern survived. Either the fern adapted to live on top of living trees to receive more sunlight, or these ferns can survive for long periods without water. It has even been theorized that the resurrection fern can stay in this dormant state for at least 100 years [4].

On average, plants' water, sunlight, and minerals are the most crucial parts of a plant's ability to perform photosynthesis, cellular respiration, and other cellular functions [8]. Photosynthesis is the process in which a plant makes glucose which feeds the plant. Through two processes light-dependent and light-independent glucose is made taking in carbon dioxide and water [9]. Aerial carbon dioxide and oxygen are vital to plants. Carbon dioxide is used in photosynthesis and oxygen in cellular respiration. Terrestrial plants also require nutrients such as nitrogen, potassium, and phosphorus, which are assimilated from the soil. However, unlike terrestrial plants, if dehydrated, resurrection ferns go into a physiological rest period using special proteins called dehydrins to lubricate the cells and keep them from dying. Another difference is that the resurrection fern gets most of its nutrients from leaf litter, not soil [8].

The stressors that plants are subjected to during their lifetime generally fall into two categories, abiotic and biotic. Abiotic factors include radiation, salinity, extreme temperatures, floods, and droughts. Factors like pathogens, including fungi, oomycetes, nematodes, and herbivores are biotic stressors. Unlike animals, which can seek shelter, plants are stationary and are exposed to the elements. As a result, plants have developed adaptive mechanisms to tolerate external factors. Plants physiologically acclimate to seasonal changes through hormones due to temperature changes or daylength. These mechanisms occur inside the cell within the cytoplasm. By using multiple transduction pathways, the stimuli are received and transferred to the transcriptional machinery in the cell's nucleus. This gives way to different transcriptional changes, such as making proteins and allowing the plant to tolerate the upcoming stressor. An example of transcriptional changes would be when the resurrection fern makes dehydrins and heat shock proteins to dehydrate the plant while maintaining cell wall integrity.

Another line of defense in plants is shown in their roots. However, the chances of the plant surviving this stressor will be a lot higher if the plant is healthy and the soil is biologically diverse. Many things can happen when stressors begin to trigger the plant, such as gene expression, cellular metabolism, and changes in growth rates. In some cases, stressors may have injured the plant so severely that they exhibit dysfunction with their metabolism, seed formation, flowering, or death. On the other hand, biotic stress is harder for a plant to detect. This is because plants lack an adaptive immune system. Despite this, plants have evolved defensive strategies in their genetic code [10].

A plant's response to stress is accompanied by electrolyte leakage. Electrolyte leakage is most found when potassium ions leave the plant cells, which are usually kept in check by the plasma membrane. The majority of the time, this is found with a reaction resulting in programmed cell death. Oxygen species (ROS) accompanies this reaction and activates the potassium ions efflux [11].

In a previous experiment, researchers measured the reaction to heat stress in desiccation-tolerant plants; the main plant was the resurrection fern. After exposing the resurrection ferns to heat stress, they measured their effects using metabolic indicators. Ferns were exposed to 50˚C in their hydrated state and 65˚C in their desiccated state. Both hydrated and dehydrated fronds started showing effects above 40˚C, but both hydrated and dehydrated ferns remained metabolically active to 50˚C. When exposed to 40˚C, there was slight discoloration after being rehydrated, but when exposed to >50˚C, there was permanent discoloration after rehydration. They conclude that the dehydrated resurrection fern was better at protecting the fern from heat stress by recovering up to 55˚C, whereas the hydrated ferns could only tolerate 40˚C [12]. This research assisted the researchers in establishing temperature parameters for this study.

The resurrection fern is tolerant to dramatic environmental changes. These plants caught NASA's attention and were taken to the international space station to be observed. NASA discovered that the resurrection fern can even "resurrect" in zero gravity. NASA also found that the fern could tolerate zero gravity due to the adaptation that the plan uses to survive. As it starts to dehydrate, the plant curls up to protect from the sun, whereas a regular plant's cells would collapse, no longer maintain healthy cytoplasm, and lose integrity. Membrane integrity is essential during desiccation. The degree of injury caused by extreme temperatures can be measured [13]. The fern also produces late embryogenesis abundant proteins or LEAs, dehydration proteins or DHAs, and heat shock proteins or HSPs to protect from the sun and maintain cell elasticity. These proteins also continue photosynthesis and aid in the resurrection of the plant once water returns [14].

Heat shock proteins (HSPs) are one of the main proteins to help the resurrection fern during dehydration. These proteins can be one of two types of HSPs, those that use ATP and those that do not. Both protect proteins from denaturing in the process of protein folding. An interesting fact is that these proteins are also found in humans, especially during the embryonic development of the nervous system. In addition, heat shock proteins are what help other proteins fold correctly. These proteins are part of the molecular chaperone system. The chaperone system helps the fern's proteins not denature due to the fern's changing shape and environmental condition. These proteins also increase elasticity in cell structure [15].

Late embryogenesis abundant proteins (LEAs) also help the resurrection fern during dehydration. They are divided into 8 subgroups LEA1, LEA2, LEA3, LEA4, LEA5, LEA6, seed maturation proteins, and dehydrin (DHN). Dehydrins and LEA2s are the specific ones that work the most in a plant's reaction and adaptation to antibiotic stress. These proteins are commonly found in the roots, stems, and other organs in a plant's growth phase. LEAs are distributed in organelles like the mitochondria, chloroplast, or the cytoplasm [16]. After significant environmental changes, LEA levels go unregulated, leading people to believe that these proteins are essential to responding to environmental stressors. Dehydrins and LEA2s are found explicitly throughout the cell during abiotic stressors that cause dehydration, such as salinity, drought, cold, heat, etc. Because the expression of many DHNs increases with the phytohormone abscisic acid (ABA), they are also known as RAB proteins. Like all LEAs, DHNs are found across most plants. All these intricate responses and adaptations by the resurrection fern open up a multitude of possibilities.

2. Materials and Methods

The first study was initiated on April 25, 2020. Four treatments were initiated by sorting 32 specimens of resurrection ferns into 4 groups of 8. Prior to experimentation, the ferns were conditioned to the imposed treatments (either dried or hydrated), which were placed into a container with an inch of desiccate to dry the tissue and the other half were under a damp paper towel which hydrated the tissue. The ferns were then exposed to −50˚C (ultra-low), 0˚C (freezer), 25˚C (room temperature), and 50˚C (forced air drier) for the duration of a week. Afterward, the ferns were reconditioned by wrapping in moist paper towels to maintain unity for recovery, and water was added and left for another week. Data was collected using a 1 - 10 scale (1 = dead, 5 = moderate growth recovery, and 10 = vigorous growth recovery) data were analyzed using an ANOVA at the 0.05 level of significance. The experiment was terminated, and the ferns were harvested for evaluation.

A second study was started, and specimens were harvested from the same live oak. The ferns were randomly split into two groups (hydrated or dehydrated). Of those groups, the ferns were evenly divided into twelve separate smaller groups. The groups were exposed to the three treatments −50˚C (ultra-low), 25˚C (room temperature), and 50˚C (forced air drier) for 48 hours. 0˚C was excluded due to space constraints. Then 100 ml of distilled water was measured and poured into an Erlenmeyer flask. The ferns were then cut at the base of the frond and each group is submerged into the flasks. Then all the filled flasks were placed in a New Brunswick Scientific shaker and left for 48 hours allowing the salt from the damaged cells to flow out of the fronds. The ferns electrolyte leakage was then measured by a conductivity meter and analyzed using an ANOVA at the 0.05 level of significance. The experiment was then terminated.

A third study was started, and new actively growing specimens were harvested from the same live oak. Three hydrated ferns were measured and then left in the forced air dryer for 7 days. Afterward, the ferns were measured again, and the water content was calculated.

3. Results and Discussion

***Study* 1.** There were significant differences when hydrated and dehydrated ferns were exposed to different temperatures. The dehydrated ferns and hydrated ferns were both exposed to −50˚C and after seven days of recovery were rated. The dehydrated ferns were measured at a level of 9 (the ferns were actively growing and 90% of foliage was green) compared to the hydrated ferns, rating at level 2 (<20% of the foliage was brown) (Figure 1). Leaf tissue from these treatments were examined at 200× power under a microscope indicating that the hydrated tissue recovery showed visible damage from the low temperatures compared to the control (Figure 2). The fronds exposed to 0˚C also exhibited a

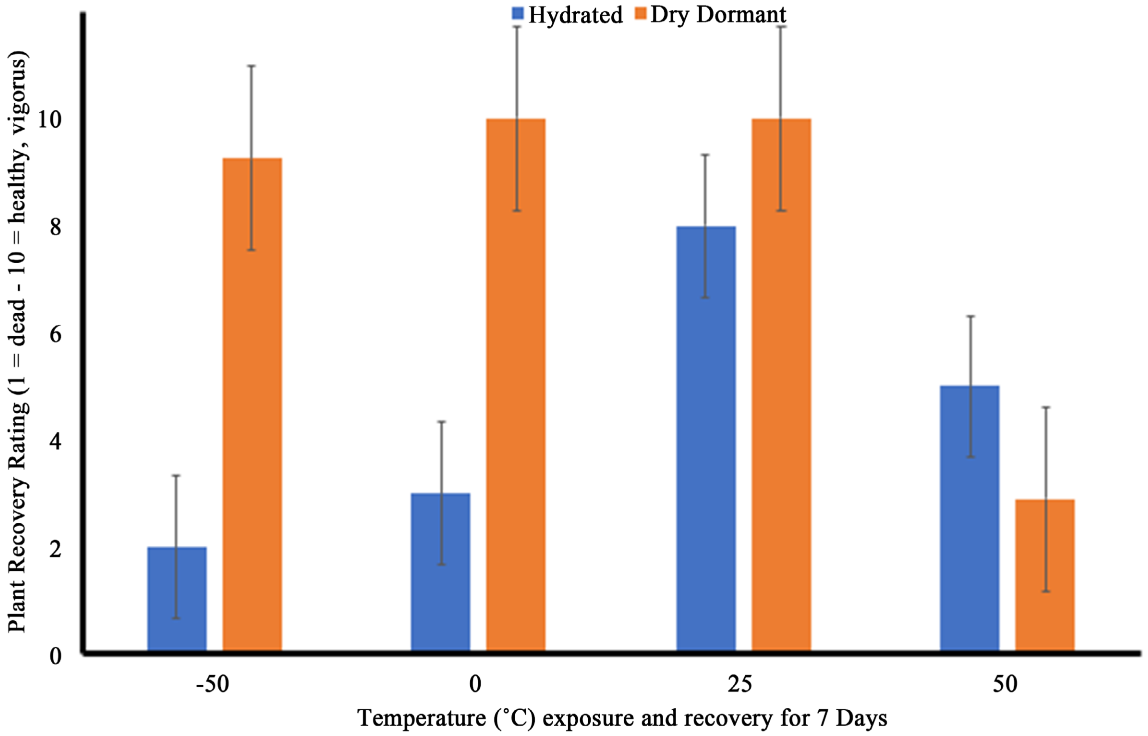

Figure 1. Exposure of resurrection fern to extreme temperature exposure after 7 days and subsequent plant recovery.

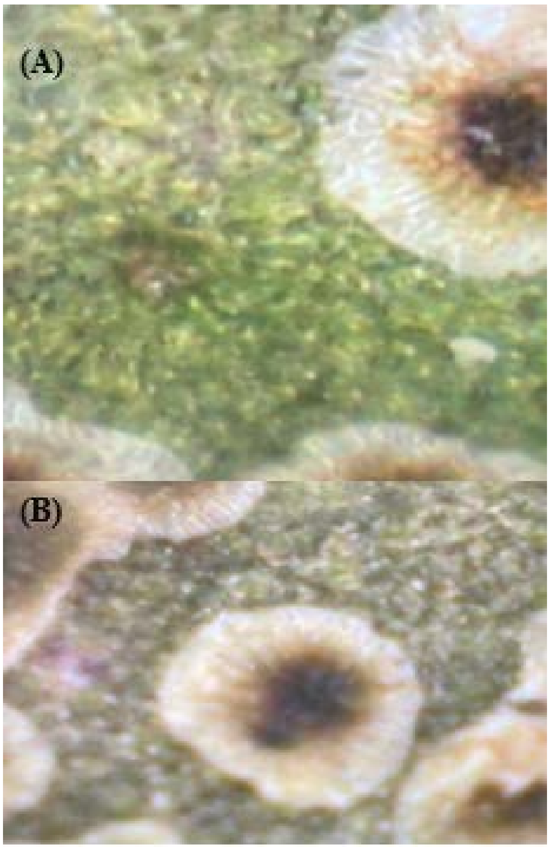

Figure 2. Two Images of a scope on the rope at 200×. (A) Hydrated resurrection fern exposed to 25˚C for 7 days. (B) Hydrated fern after exposed to −50˚C for 7 days.

significant difference between the desiccated ferns which were at a rating of 9 and the hydrated which were at the level of 2. There were no differences between the moisture levels for ferns exposed to 25˚C both moisture levels were measured at a healthy level, which is expected since the temperature was in the optimum temperature range. Lastly, there was a difference between moisture levels exposed to 50˚C. The dry specimens were measured at a level of 3 (less than 30% foliage was brown) and the hydrated was measured at a level of 6 (the ferns were actively growing and 60% of the foliage was green); however, compared to the other environments, the dehydrated ferns did not recover as well.

***Study* 2.** There were significant differences in electrolyte leakage between the hydrated and dehydrated ferns exposed to different temperatures. Also, while preparing for the experiment all the ferns rehydrated even after the roots had been cut; thus, the ferns have the ability to survive without roots. The ferns exposed to −50˚C had significant differences in moisture levels. The dehydrated fronds exposed to −50˚C leaked an average level of 25.6 uS/cm versus the hydrated specimens that leaked an average level of 138.1 uS/cm (**Figure 3**). The ferns that were exposed to 25˚C had significant differences in their moisture levels. The dehydrated ferns leaked an average of 30.55 uS/cm versus the hydrated ferns which leaked an average of 2.1 uS/cm (**Figure 3**). The ferns exposed to 50˚C degrees had significant differences between moister levels. The dehydrated ferns measured at an average of 76.6 uS/cm versus the hydrated ferns which averaged at a level of 98.1 uS/cm (**Figure 3**). However, there was also a specimen that leaked significantly more showing that there can be cases where certain specimens can take more damage.

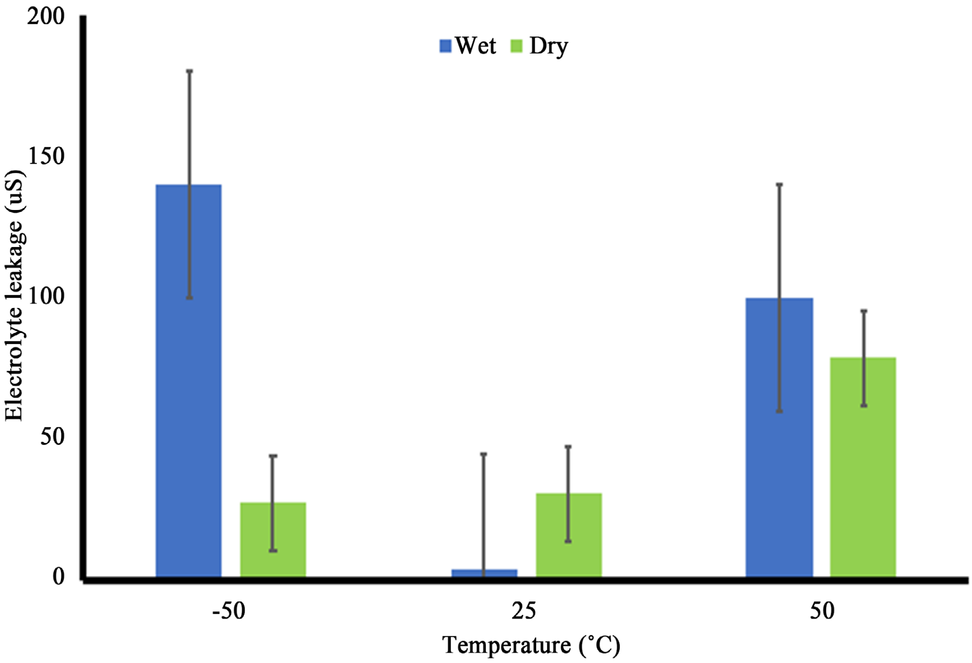

Figure 3. Electrolyte leakage of resurrection fern exposed to extreme temperatures dry or hydrated.

***Study* 3.** The actively growing resurrection ferns were harvested to determine the percent of water weight in living tissue. The average water content in resurrection ferns (consisting of resurrection fern fronds, rhizomes, and roots) was determined to be greater than 59%.

4. Conclusion

In conclusion, the resurrection fern is overall more tolerant to different temperatures when the tissue is in its desiccated state. The dehydrated ferns exposed to 0˚C and −50˚C notably were similar to the control group. This study determined that resurrection ferns were found to be resistant at extreme conditions, especially in a dehydrated state. The ferns were more tolerant to colder temperatures than warmer temperatures. This could be due to proteins being denatured and dehydrated being unable to decrease temperature through transpiration. Lastly, it was shown that the hydrated fronds were about 2.3 times bigger than the desiccated fronds.

Application to Society

Understanding the adaptive mechanisms of resurrection ferns could be instrumental in the transformation of domesticated food crops, space exploration, and stopping world hunger. Identifying and transferring desiccation tolerant genetics could have beneficial human impacts around the world. If this study becomes a reality and cell integrity can be transferred to essential crop plants, the result could lead to plants that are tolerant of drought, temperature extremes, and loss of resources needed for survival. A possible way is through a transgenic transformation where it is possible to transfer a genome from one plant to another. This would allow food-producing plants to survive longer without water, making extended trips for space travel possible. In the foreseeable future transgenic plant that is tolerant in a lunchbox-like planter where plants could be hydrated or produced.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] NASA (2021, February 17) The Causes of Climate Change. Global Climate Change: Vital Signs of the Planet. https://climate.nasa.gov/causes/

[2] Dostler, G. (2017, March 1) 7 Reasons Why Plants Are Valuable and Important. Plantscapers. https://plantscapers.com/7-reasons-why-plants-are-valuable-and-important/

[3] Sprunt, S.V., Schneider, H., Watson, L., Russell, S.J., Navarro-Gomez, A. and Hickey, R.J. (2011) Exploring the Molecular Phylogeny and Biogeography of Pleopeltis polypodioides (Polypodiaceae, Polypodiales) Inferred from Plastid DNA Sequences. *Systematic Botany*, **36**, 862-869.

[4] Taylor, A. (2017, May 18) Resurrection Fern (*Pleopeltis polypodioides*)—Island Ecology 2017. Island Ecology. https://sites.google.com/site/islandecology2017/terrestrial-flora/resurrection-fern

[5] Stuart, T.S. (1968) Revival of Respiration and Photosynthesis in Dried Leaves of *Polypodium polypodioides*. *Planta*, **83**, 185-206. https://doi.org/10.1007/BF00385023

[6] U.S Forest Service (n.d.) What Are Ferns? https://www.fs.fed.us/wildflowers/beauty/ferns/what.shtml#:%7E:text=Similar%20to%20flowering%20plants%2C%20ferns,exemplified%20by%20the%20walking%20fern

[7] JRank (2021) Ferns—General Characteristics. https://science.jrank.org/pages/2679/Ferns-General-characteristics.html#:%7E:text=However%2C%20there%20are%20two%20principal,leaves%20or%20on%20specialized%20branches

[8] Gailliard, T. (2018) What Are Three Things Plants Need to Live? Sciencing. https://sciencing.com/what-are-three-things-plants-need-to-live-13428190.html

[9] Peterson, S. and Peterson, B. (Directors) (2014) Photosynthesis and the Teeny Tiny Pigment Pancakes. https://www.youtube.com/watch?v=uixA8ZXx0KU&vl=en

[10] Gull, A., Ahmad Lone, A. and Ul Islam Wani, N. (2019) Biotic and Abiotic Stresses in Plants. In: de Oliveira, A.O., Ed., *Abiotic and Biotic Stress in Plants*, IntechOpen, London, 1-5.

[11] Demidchik, S.D., Medvedev, S.S., Pozhvanov, G.A., Sokolik, A. and Yurin, V.V. (2014, February 11) Stress-Induced Electrolyte Leakage: The Role of K^+-Permeable Channels and Involvement in Programmed Cell Death and Metabolic Adjustment. *Journal of Experimental Botany*, **65**, 1259-1270. https://doi.org/10.1093/jxb/eru004 https://pubmed.ncbi.nlm.nih.gov/24520019/

[12] Hasenstein, K.H. and John, S.P. (2020) Desiccation Mitigates Heat Stress in the Resurrection Fern, *Pleopeltis polypodioides*. *Frontiers in Plant Science*, **11**, Article ID: 497731. https://doi.org/10.3389/fpls.2020.597731

[13] Antony, A. and Leslie, A.G. (2011) Degradation of Polymeric Membranes in Water and Wastewater Treatment. In: Basile, A. and Pereira Nunes, S., Eds., *Advanced Membrane Science and Technology for Sustainable Energy and Environmental Applications*, Woodhead Publishing, Sawston, 718-745. https://doi.org/10.1533/9780857093790.5.718

[14] Krieg, C. and Saunders, S. (2016) Ferns in space. Palmetto, Florida.

[15] Miller, D.J. and Fort, P.E. (2018) Heat Shock Proteins Regulatory Role in Neurodevelopment. *Frontiers in Neuroscience*, **12**, Article No. 821. https://doi.org/10.3389/fnins.2018.00821

[16] Chen, Y., Li, C., Zhang, B., Yi, J., Yang, Y., Kong, C., *et al.* (2019) The Role of the Late Embryogenesis-Abundant (LEA) Protein Family in Development and the Abiotic Stress Response: A Comprehensive Expression Analysis of Potato (*Solanum tuberosum*). *Genes*, **10**, Article No. 148. https://doi.org/10.3390/genes10020148 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6410179/