Proector of the Seeds: Seminal Reflections from Southern Africa

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Despite their marked geographical and cultural diversity, the peoples of Africa are bound together by concerns about food security and the vagaries of rainfall across the continent’s extensive terrain, much of which is arid or semi-arid. This makes the scientific study of seeds and their storage an imperative. I became convinced of this scientific mandate even as a graduate student at the University of Natal in Durban in the late 1960s where, under the guidance of Trevor Villiers, I metamorphosed from an animal-oriented biochemist into a seed-focused cell biologist.

To most people, a seed is a dry structure that can be maintained in a desiccated condition in a state of suspended animation until provided with water and other conditions that will promote germination. These traits define “orthodox” seed behavior. Maize (corn), which produces orthodox seeds, is the staple crop of much of Africa, yet it is ill-suited to the drought-prone conditions that prevail in many regions, where it is cultivated in preference to the native cereal, sorghum.

Annual production of maize is important not only for food security, but also in providing seeds for planting in following seasons. Unfortunately, the crop is frequently jeopardized by droughts. The threat to the crop is exacerbated by seed storage under warm, high relative humidity conditions that can drain seeds of their vigor and viability, while encouraging fungal growth in the seeds. My doctoral work on maize seeds aimed to characterize the course of rapid deterioration that inevitably occurs under these poor seed-storage conditions. I concentrated on the root cap of the seed embryo. After germination, the integrity of this structure is essential to protect the tip of the root as it grows through the sharp, abrasive soil.

Radical Fungi

There were several exciting outcomes from those investigations. The first was the original microscopic characterization in plant cells of lysosomal vacuoles—fluid-filled vesicles collectively containing enzymes capable of breaking down all other intracellular constituents. A second discovery was that cells that form the root cap self-destruct by autolysis in the final phase of their developmental program (a process called apoptosis, or programmed cell death) and are sloughed at the cap surface. The work also showed that the events involved in apoptosis are accelerated when seeds are poorly stored and that intracellular membranes are the primary loci of degeneration.

Membranes are pivotal for compartmentalizing intracellular functions. They also provide the selective barrier between the cell and its surroundings. Membrane breakdown is a key factor in cell debilitation and death. For seeds, that translates into a loss of viability. Then, as now, the generation of free radicals within the cells of dry seeds in storage is considered to be a major cause of deterioration of membranes and other cellular structures.

On the premise that membrane damage is caused by free-radical activity, Norman Pammenter, my husband and major research collaborator, and I had an inspiring discussion with a Hungarian animal physiologist, K. Molnár, about his work on the efficacy of cathodic protection in extending the lifespan of mice.

Consequently, we stored maize seeds under deteriorative conditions, but in a static electric field. The results, published 30 years ago in this journal, showed that the application of cathodic protection had a dramatic effect in extending seed lifespan. That outcome could be attributable to quenching of free radicals. With hindsight, however, another interpretation is also possible: The efficacy of the treatment resulted from its adverse effects on fungi within the seeds.

With the help of two graduate students, David Mycock and Michelle McLean, my laboratory became active in seed fungus research. The fungi in question are xerotolerant—they survive the dry conditions within stored orthodox seeds. They also produce mycotoxins, which include some...
of the most carcinogenic materials known (for example, the aflatoxins).

Working with maize, we showed that individual fungal species, which continue to be metabolically active under storage conditions of high relative humidity, replace each other in a process of succession, all the while causing increasing damage within the seeds. We also established that xerotolerant fungi can be transmitted asymptotically through the growing maize plant, even infecting the next seed generation. Of practical importance in rural Africa was the demonstration that thermotherapy—pre-storage immersion of maize grains for short periods in hot water—can substantially reduce fungal loads in maize seeds. Once the seeds are redried after thermotherapy, their potential to be stored for long periods is considerably improved. The reduced mycotoxin levels also make the seeds safer for consumers.

My group’s most recent foray into xerotolerant seed fungi centers on the unique gymnosperm of the Namib Desert, Welwitschia mirabilis, which is potentially endangered by a seed-associated, ultradesiccation-tolerant fungal species. This plant, which produces only two leaves throughout its long life-span and a root that grows down to the deeply situated water table, provides the only islands of refuge for a variety of small desert animals. Water droplets condensed from nocturnal sea fog run down the plant’s long, downward curving leaves, providing the moist environment essential for the animals’ survival. Solving the seed fungus problem of W. mirabilis, therefore, is of major importance to the survival of the Namib ecosystem.

The Unstorables
In the 1980s my collaborator husband and I, with a succession of our students, began investigations of wet, recalcitrant seeds. Such seeds exhibit unorthodox traits because they cannot withstand dehydration and remain desiccation-sensitive throughout their development and after harvest. The term “recalcitrant,” defined as “obstinate disobedient,” was first applied by seed scientists to describe the responses of seeds that could not be stored under the conventional low-temperature and low-relative humidity conditions used for orthodox seeds. The category includes seeds of commercially important plants, including those that produce rubber and cocoa, many tropical and subtropical trees, a few temperate species, and a wide spectrum of plants heavily used in Africa for traditional medicine.

Plants in the last-mentioned category include many trees, shrubs, and nonwoody (herbaceous) species of which the bark, leaves, seeds, roots, and bulbs are commodities, collectively worth U.S. $45 million annually. More than 70% of the South African population relies on traditional medicine, and current estimates are that around 4000 tons of plants or plant parts are traded annually in the Durban area alone in traditional medicine (muthi) markets.

Many of the plants used for traditional medicine face a double threat—their recalcitrant seeds are short-lived and hard to store, and the plants are overharvested. The pepper-bark tree (Warburgia salutaris), for example, has been harvested to extinction in the wild in South Africa.

When we first turned our attention to recalcitrant seeds, little was known about why they could not be dehydrated and why, even if well hydrated, the recalcitrant seeds of most species could be stored only for periods too brief to be useful for long-term conservation of genetic resources. In subtropical Durban, on the eastern seaboard of southern Africa, we were well placed to study seed recalcitrance, having local access to appropriate plant species and the sophisticated laboratory infrastructure necessary to explore the phenomenon.

Using electron microscopy and biochemical analyses, we first showed that highly recalcitrant seeds undergo all the metabolic changes characteristic of the initiation of germination. We showed further that this metabolism continues during the early stages of dehydration, until intracellular damage becomes overwhelming. When Jill Farrant subsequently joined us as a graduate student, we demonstrated that recalcitrant seeds, when stored in hydrated conditions—a humidity high enough to allow the seeds to retain a concentration of water on a par with what it was when the seeds were shed from the tree—become increasingly desiccation-sensitive as the cellular events of germination progress. Without an extraneous water supply, the seeds will begin to deteriorate.

These were definitive discoveries, explaining why visible initiation of germination while seeds were in storage was not merely a nuisance, but was lethal for recalcitrant seeds. A seed that has germinated to the point of requiring additional water will not retain viability unless immediately planted, and is not worth storing. That finding only redoubled our efforts to find new ways of storing these recalcitrant seeds.

Seed Taming
Recalcitrant seeds are not only desiccation-sensitive, but also metabolically active. In contrast, orthodox seeds, owing to their dry state, are metabolically quiescent. Lowering the water content to a level that would preclude germination but facilitate vital metabolism has been suggested as a way to extend the life-span of recalcitrant seeds in hydrated storage. However, Daniel Côme and Françoise Corbineau of the Université Pierre et Marie Curie in Paris, and we, have independently shown that this practice of partial dehydration curtails the seeds’ storage life-span.

Current work by graduate students Déon Erdey and Sharon Eggens in our laboratory suggests that slight dehydration stimulates the onset of germinative metabolism, thereby shortening the window of time before additional water is required by the seeds. To optimize storage life-span, just the opposite needs to happen: The onset and progression of germinative metabolism need to be delayed.

Recalcitrant seeds are so-named for a reason. Storage at lowered temperatures might seem an obvious answer, because metabolic rate is slowed in the cold, but many species of tropical and subtropical origin are sensitive to chilling. And even when all the conditions for short- or medium-term hydrated storage...
have been optimized, most species of recalcitrant seeds face a further limiting factor—
fungal infections.

Seed-associated fungi are ubiquitous and pose a prodigious problem: They use seed tissues as their source of nutrition. As a result, the seeds rapidly weaken and die. With graduate student Claudia Calistru and others, we showed that if the seed’s fungal load can be reduced or eliminated, then seed storage life-span can be doubled or even quadrupled, depending on the species. Although promising, even this advance is not enough for useful long-term storage of highly recalcitrant seeds.

We also have been pursuing another strategy for halting germinative metabolism in order to increase storage times: deep-freezing. There has long been a consensus that achieving and maintaining the deep-frozen state by cryostorage—usually in liquid nitrogen at –196°C—is the only solution for long-term storage of recalcitrant seeds.

But how can this be achieved practically? Recalcitrant seeds—whether coconuts or the “pips” of a litchi, mango, or avocado pear—are generally large. These seeds also are “wet.” Such large, hydrated living structures will not be able to withstand the effects of freezing; the ice crystals wreak lethal havoc close to those of so-called nonfreezable water. Simply put, this is the water that is closely associated with intracellular structures. It does not freeze in any standard sense. In contrast, most of the water within cells occurs as solution water, also called freezable water.

Many of the specific parameters for cryopreservation of excised axes were elucidated and quantified during a collaboration with Christina Walters of the (then) U.S. Department of Agriculture National Seed Storage Laboratory in Fort Collins, Colorado. Together we confirmed that metabolism-linked damage—as opposed to desiccation damage, which occurs when the structure-associated, nonfreezable intracellular water is perturbed—is the basis of seed death during slow dehydration of recalcitrant material. Joining by our then-graduate student, James Wesley-Smith, many of the intricacies of axis survival in relation to freezing rate, water concentrations attained, and freezing rate have been—and are still being—resolved.

Axes of temperate seed species have so far proved better able to withstand the procedural “insults” of cryostorage than have those of tropical species. These “insults”—axis excision, application of antifungal compounds, dehydration of an essentially desiccation-sensitive structure, plunging into liquid nitrogen, and subsequent thawing and rehydration—would constitute a formidable challenge to any living organism. Nevertheless, encouraging progress has been made, for example, by our colleague Joseph Kioko, whose efforts as a graduate student facilitated drying and successful cryostorage of the seeds of the pepper-bark tree, one of the most sought-after endangered medicinal plant species in southern Africa.

It is not sufficient that axes merely survive cryostorage: They must ultimately yield growing plants that are phenotypically, genotypically, and physiologically indistinguishable from those grown directly from newly harvested seeds. Among the challenges here are to develop techniques for successful rehydration of axes and for the promotion of shoot production after cryopreservation. We also are developing synthetic seeds—called synseeds—whose individual axes are encapsulated in a gel to reconstitute seedlike structures. This work, performed with postdoctoral fellow Rosa Perán, is in its early stages, and could lead to material that is more easily handled for planting programs.

In time, we hope to offer cryobanking services for recalcitrant-seeded species in Africa. It would be our way of combating the specter of genetic erosion and extinction of the continent’s most valuable and sought-after plants.

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GLOBAL VOICES

OF SCIENCE: Protector of the Seeds: Seminal Reflections from Southern Africa
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Science 307 (5706), 47-49.
DOI: 10.1126/science.1108429