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Bibliographic review

The tolerance of seeds to drying and its implication for germplasm conservation

La tolerancia de las semillas a la desecación y su implicación en la conservación del germoplasma

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ABSTRACT: This paper summarizes researches focused to study seed desiccation tolerance and ways to preserve them. The investigations it contains are related to the properties of the solid state of the cytoplasm of dried tolerant seeds. Also, characteristics of desiccation sensitive seeds, and the general procedures for their long-term conservation are presented. Reference is made, considering different research papers, to the conceptual model that incorporates spatial and temporal effects of water loss to explain the cellular compression as damage origin, and the protection of dry matter reserves to the stability of solid cytoplasm and seed longevity.

Key words: cryopreservation, longevity.

RESUMEN: Este trabajo resume investigaciones enfocadas a estudiar la tolerancia de las semillas a la desecación y las formas establecidas para preservarlas; así como las propiedades del estado sólido del citoplasma de las semillas tolerantes desecadas. También, incluye las características de las semillas sensibles a la desecación y el procedimiento general para su conservación a largo plazo. Se hace referencia, a partir de diferentes trabajos, al modelo conceptual que incorpora los efectos espacial y temporal de la pérdida de agua para explicar la compresión celular como origen del daño por desecación, y la protección que ejercen las reservas de materia seca a la estabilidad del citoplasma solidificado y la longevidad de las semillas.

Palabras clave: criopreservación, longevidad.

INTRODUCTION

Germplasm conservation banks have as an essential function the maintenance of seed viability to reduce the frequency of regeneration of the original sample, thus reducing costs and risks of genetic erosion (1).

Seeds that tolerate extreme desiccation and low temperatures can apparently survive at least 100 years, if conventional freezer conditions (-18 to -20 ºC) are used for their preservation. Currently, there are about 1750 seed banks in the world that conserve about six million accessions; however, not all plants produce seeds, or seeds that survive freezing conditions (2).

Longevity expresses the retention of viability and vigor of a seed lot during storage (3,4), and different laboratories have accepted as a criterion for the evaluation of longevity the P50 value, defined as the storage time in which the germination of a lot decays to 50 % of its initial value (4,5).

Seed survival time (longevity) depends on storage conditions, i.e., relative humidity and temperature (6) and on the inherent properties of the species compared to others (5).

This paper presents results obtained in the last two decades in relation to the fundamental characteristics of seeds tolerant and susceptible to desiccation, and the strategies that have been developed for their conservation, so it can be used as a reference by specialists who wish to update and deepen in this subject.

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DEVELOPMENT

Desiccation-tolerant (orthodox) long and short-lived seeds

Desiccation-tolerant seeds are identified as orthodox seeds (7) because they can be stored for predictable periods under controlled conditions (8). These seeds can survive in a dry state for considerable periods of time and store successfully at low water contents and temperatures below 0 ºC (-18 to -20 ºC) (9). During maturation, dehydration-tolerant seeds undergo a natural dehydration process, in which they lose most of their tissue water until they reach equilibrium with the relative humidity of the surrounding medium.

Dried organisms differ from hydrated organisms in that they have solidified cell cytoplasm as opposed to the fluid cytoplasm of hydrated organisms (10). To understand the stability of the solid state of seeds and its relationship to longevity, a number of studies have been conducted on the stability of solid substances.

Crystalline solids are thermodynamically stable because the very close approximation between molecules minimizes the surface area, and the molecules have few places to flow. In contrast, solids formed by drying or cooling, in which the molecules are not arranged in an aligned manner, constitute porous matrices referred to as “amorphous solids” and the temperature at which they form (from a fluid) is called the glass transition temperature. Moreover, because they are irregularly aligned, the molecules will have some mobility (11); therefore, the pores are not thermodynamically stable, and the solidified structure of the porous matrix relaxes over time towards more efficient packing (12).

In the dry state, biological materials have the characteristics of amorphous solids, as they have considerable pore spaces, being formed by large molecules that do not compress well; and because of this condition they exhibit glassy brittleness, which means that they change abruptly from solid to fluid, in a narrow range of temperatures (10).

During drying, the embryonic cytoplasm of seeds tolerant to desiccation solidifies with the characteristics of amorphous solids, and this condition of the solidified cytoplasm is called vitreous state or vitreous matrix.

Studies on the relaxation velocity of the vitreous states of seeds have shown that there is considerable molecular mobility even under cryogenic conditions and confirm the practical verification that all solids maintain some molecular movement (12). It has been pointed out that the kinetics of seed aging is regulated by the tendency for movement in the molecular structure of the solidified cytoplasm (11-13).

In order to find the relationship between molecular mobility and the physiological activity of seeds, studies have been carried out with different types of analysis, including dynamic mechanical analysis (DMA). This analysis allows measuring the relationship between an applied force and the structural deformation produced on the material that receives it, so that it allows detecting differences in the structural stability of the materials (12); therefore, it is a valuable tool to quantify the structural stability and molecular mobility of the vitreous states of the seeds (12,14).

DMA was applied to study molecular movements in dry cotyledons of pea (*Pisum sativum*) and demonstrated the existence of intramolecular movements of the vitreous matrix of cotyledons, and that the nature and extent of these movements vary considerably with humidity and temperature (11).

Research focused on studying the longevity of seeds in storage has allowed finding differences between plant families. Thus, some families, such as the *Apiaceae* and *Brassicaceae* are characterized by short shelf life, whereas the seeds of the *Malvaceae* and *Chenopodiaceae* are long-lived, which gave rise to the hypothesis that individual species have a characteristic storage life potential (5).

DMA was also used to study the solid states of the embryonic axes of pea and soybean (*Glycine max*), long- and short-lived seed species in storage, respectively. The analysis made it possible to determine that in the solid state of the cytoplasm, vibrational and rotational molecular movements occur; and that, at temperatures between 25 and 100 ºC, diffusive molecular movements occur in the embryonic axes that produce the transition of the material from the solid to the fluid state (10). When comparing the two species, it was detected that the solid matrices of soybean embryonic axes showed evidence of high presence of diffusive motions and the properties of brittle glassy matrices, suggesting that they may be prone to aging; whereas the structures within pea axes showed lower mobility (10). The results of these analyses are consistent with the longevity characteristics of these species, referred to above.

Preservation of desiccation-tolerant seeds

Standards for genebanks state that original and duplicate safety samples should be stored under long-term conditions (base collections) at a temperature of -18 ± 3 °C and relative humidity of 15 ± 3 %. For medium-term storage (active collections), the samples are stored refrigerated between 5 and 10 °C, with a relative humidity of 15 ± 3 % (1).

It is estimated that dried orthodox seeds, which survive for 25 to 50 years under refrigerated conditions, can survive for a period of 100 to 200 years in a freezer (2). Moreover, cryopreservation has been shown to extend the longevity of dried seeds compared with storage in traditional freezers (-18 to -20 °C) (15). However, the degree of seed desiccation plays an important role in the response to cryopreservation. Results have been found in which seeds with a moisture content between 5-13 % showed no damage from ultra-low temperatures, while those with moisture contents between 13-16 % showed damage (16).

Desiccation-sensitive seeds (recalcitrant, exceptional)

The term recalcitrant originated to describe seeds whose storage life was not predictably increased by reduced water content and ambient temperature (8), so the term became descriptive for desiccation-sensitive seeds of limited longevity.

The desiccation-sensitive condition has been associated with crop ecology. Species that produce sensitive seeds are mostly, but not exclusively, confined to uniform habitats that favor seed germination and seedling establishment (17). Dispersal mechanisms and post-harvest physiology are thought to have evolved to ensure that germination occurs when conditions are favorable for plant establishment, prior to loss of viability; this may explain why recalcitrant seeds are less common in markedly seasonal climates than in the humid tropics.

Sensitive seeds lack the late developmental stage of orthodox seeds, lacking or not expressing the genetic information that allows the development of desiccation tolerance. At the time of fruit harvest, they are metabolically active, hydrated and cannot be stored at temperatures below 0 ºC. Desiccation-sensitive seeds show considerable variability among species and within individual species with respect to the magnitude of water loss they can tolerate (9).

Recently, desiccation-sensitive seeds have been identified as “exceptional seeds,” considering that, in the context of germplasm banking and cryobiotechnology, the term recalcitrant can be confusing, as it applies to seeds with complex requirements for germination that involve separate and repetitive periods of wetting at alternating temperatures to stimulate rudimentary embryo development (2).

For woody species, a method has been developed to predict sensitivity to desiccation that establishes a mathematical relationship which considers the dry mass of the seed coat structure (endocarp and testa) and the dry mass of the total seed. If the result of this calculation, called probability of desiccation sensitivity (P), is greater than 0.5, the species appears to be sensitive to desiccation (18-19).

Preservation of desiccation-sensitive seeds

The only possible method for long-term conservation of desiccation-sensitive germplasm is cryopreservation (20), which consists of storing the material at the ultra-low temperature of liquid nitrogen (-196 ºC). For the success of cryopreservation, it is essential to avoid the formation of ice inside the cells, causing the freezable water present in the cells to vitrify and form an amorphous solid (non-crystalline) of very high viscosity (vitreous state) (9), similar to the solid state naturally acquired by the cytoplasm of seeds tolerant to desiccation.

In relation to the drying of sensitive seeds, it has been found that material that dries quickly (in the term from minutes to a few hours) can survive (in the short term) to materials with lower water content, but that dry slowly. That effect is more pronounced as drying becomes more rapid, but most recalcitrant seeds are too large to obtain the required drying rates. In these cases, excision of the embryo or embryonic axis provides an explant of a size that does allow rapid drying, and partially dehydrated embryonic axes can generate seedlings (21).

Zygotic embryos and embryonic axes can be dehydrated by rapid drying by placing the explants in a desiccator with activated silica gel, through which air is circulated by a computerized fan; however, it should be noted that the speed of drying differs between explants of different species, seed lots and between tissues that constitute individual embryos (22).

In a study of embryos of four recalcitrant seed species, it was determined that their morphology and anatomy determine the drying characteristics of the different tissues that compose the explant, and hence the survival to cryopreservation; so that the intensity of drying and its duration also interact with the morphology and size of the embryo (or embryonic axis), to produce (or prevent), its survival (22).

Embryos or embryonic axes of sensitive seeds also require cooling to occur very rapidly, because in the temperature range just below 0 to -80 ºC ice crystal formation and growth (in insufficiently dehydrated cells) is possible. For this reason, most cryopreservation protocols for cryopreservation of complex multicellular tissues include very rapid cooling and return to room temperature rates. The fastest cooling rate is achieved by placing explants in what has been termed nitrogen slush, which forms at -210 ºC (23).

Conceptual model incorporating the spatial and temporal effects of water loss

In recent years, research describing seed characteristics in storage has advanced using a conceptual model that quantifies the cellular response to desiccation stress, considering spatial and temporal scales. According to this model, the primary response to water stress is considered spatial by assessing the amount of water leaving the cells in terms of volume change. The model also links protection from damage to the effect of the accumulation of molecules of solid reserve substances, which form a stabilizing structure (24).

Dehydration tolerance and seed longevity are acquired during the middle and late stages of embryo development because embryo cells accumulate specific compounds that are associated with the ability of cells to tolerate extreme water stress, such as low molecular weight antioxidants; complex carbohydrates, such as oligosaccharides; late embryogenesis abundant proteins (LEAs); and heat-shock proteins (HSPs) (12,25).

The model assumes that the spatial characteristics that the solidified cytoplasm acquires at the moment of desiccation will determine the time of conservation of its solid state, and the longevity of the seeds (24). The same was used to identify the minimum critical level of cellular dry matter accumulation required for proper desiccation tolerance of *Pritchardia remota* (a desiccation-sensitive tropical palm). It was shown that developing seeds, or embryos, must acquire at least 35 % reserve material to avoid a lethal reduction in cell volume during drying (26).

A similar relationship was found in studies with tobacco (*Nicotiana tabacum* L.) seeds, cultivar 'SS-96', in which it was observed that seeds harvested before reaching 35 days post anthesis (35 DAA) showed a decrease in desiccation tolerance and, as a consequence, a decrease in longevity after cryopreservation (27). Congruently, in another study on the characteristics of the seeds of five mulberry (*Morus alba* L.) varieties, it was found that their desiccation sensitivity index (P) was less than 0.5, characteristic of tolerant (orthodox) seeds, and that more than 60 % of the total dry matter was part of the nutritional reserves (embryo-endosperm) (28).

Dehydration tolerance of resurrection angiosperms (plants that remain viable despite considerable dehydration and recover metabolic activity when water becomes available) is based on a spectrum of mechanisms that accompany drying in a manner similar to that of orthodox seeds (29).

In this regard, there are an estimated 153 species of angiosperms, commonly referred to as resurrection plants that survive the loss of up to 95 % of cellular water content for prolonged periods of time and then recover tissue metabolic activity within a rehydration time of 24 to 72 hours (30).

Active and reversible folding of the cell wall and replacement of water in the vacuoles by nonaqueous substances are considered to be two possible general mechanisms used by desiccation-tolerant angiosperm plants to avoid mechanical stress during desiccation (31). These protective mechanisms are consistent with the spatial and temporal model with respect to the criterion that the compressed molecules form a structure that protects the stability of the solidified cytoplasm and contributes to seed longevity (24).

Taking into account the spatial and temporal conceptual model, studies have been conducted on the mechanisms that control the rate of aging and variations in longevity between cell types. Three types of interactions between the structural conformation of dry cytoplasm and aging have been proposed: 1) cells that contain no chlorophylls and store few lipids may exhibit long storage life and aging likely occurs due to auto oxidative processes when the solid state of the cytoplasm relaxes; 2) cells with active chlorophyll may die rapidly, as they are prone to oxidative stress originating from photosynthetic pigments in the absence of metabolic water, and 3) cells lacking chloroplasts but having high lipid content may die rapidly during storage at -20 °C, possibly because at that temperature lipids crystallize, shrink, and destabilize the glassy matrix (25).

Intermediate seeds

So-called intermediate seeds have more characteristics of desiccation tolerant seeds than recalcitrant seeds, but their storage life is not increased by temperature reduction (32).

The intermediate status can manifest itself in different ways, including 1) seeds that can tolerate drying at lower water contents than recalcitrant seeds, but not as low as orthodox seeds; 2) show anomalous longevity at temperatures between +10 and -30 ºC; and 3) are short-lived, regardless of how they have been dried or cooled. The existence of an intermediate category of postharvest physiology demonstrates the natural variation of seeds in response to water loss (24).

The anomalous longevity at temperatures between +10 and -30 ºC appears to stem from crystallization of triacylglycerols (TAGs), because the lipid-rich cytoplasm is a composite material, with different thermal properties. Shrinkage of lipid bodies produces destabilizing voids in the cytoplasmic matrix (24) and allows the entry of large molecules of reactive oxygen species (ROS). TAGs are a common feature of cells of characterized germplasm of intermediate behavior. In 80 % of the species characterized as intermediate, the TAG content in seeds exceeds 20 % and exceeds 40 % in 57 % of these species (25).

CONCLUSIONS

- The research presented shows that the procedures used for the long-term conservation of seeds, whether orthodox seeds of short or long life, as well as the embryonic axes of recalcitrant seeds, have in common with the protocols used for the cryopreservation of vegetative materials the need to achieve the solidification of the cell cytoplasm, to slow down the movements of the molecules that participate in the chemical reactions characteristic of aging and the loss of viability.
- Works reviewed show that there are differences between species in the characteristics of the molecular movements that occur in the solidified cytoplasm with temperature variation, which is congruent with the theory that considers longevity as an inherent property of the species. The existence of molecular movements, even at cryogenic temperatures, shows that, although cryopreservation can significantly extend the conservation period for many species in relation to other procedures, current conservation methods require the control of the viability of the conserved material and the renewal of the germplasm under environmental conditions to guarantee its preservation. Currently, there are no conservation methods that guarantee the preservation of germplasm in perpetuity, and therefore, over time, its renewal is necessary.

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