Popular article

STORAGE PROBLEMS OF RECALCITRANT SEEDS & THEIR CONSERVATION

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Introduction

A seed is a little embryonic plant that is wrapped in a seed coat, which normally contains some stored nourishment. It is the most important agricultural input with farmers, breeders and seed companies all relying on it. Based on desiccation tolerance and storability, seeds are categorized as orthodox and recalcitrant seeds. In *ex situ* conservation, orthodox seeds (desiccation-tolerant seeds) are those, which resist drying and freezing. The ability of orthodox seeds to endure drying and freezing varies; some seeds have been observed to be more sensitive than others. These are often long-lived seeds that may withstand dry-to-moisture ratios as low as 5% without suffering any harm. Furthermore, with low moisture content and cold conditions, orthodox seeds have a longer life. *Ex-situ* conservation of orthodox seeds is thus not much problematic. Seeds from most annual crops, biennial crops and agroforestry species are examples of orthodox seeds like *Capsicum annum, Citrus aurantifolia, Hamelia patens, Pisidium guajava, Anacardium* etc. Whereas seeds that are resistive to drying and freezing in *ex situ* conservation are known as recalcitrant seeds (desiccation sensitive seeds). They cannot be stored for extended periods like conventional seeds since their viability is lost (Chin, 1978). Recalcitrant seeds are often enormous in size and may be damaged if they are dried below 20-30 percent relative moisture content. *Ex-situ* conservation presents a challenge particularly in recalcitrant seeds because of high moisture content, which promotes microbial growth and causes seed decay quickly. Trees and shrubs are recalcitrant species, including Avocado, chocolate, coconut, mango, rubber, tea, etc. Furthermore, these seeds have a short lifespan, ranging from a few weeks to months.

Storage Problem

As recalcitrant seeds have a short life period, both short and long-term storage poses significant challenges. Perennial plants take at least three years to produce flowers and set seeds, therefore seed production and multiplication take a lengthy time. As a result, seed production and distribution are unpredictable. One of the internal physical characteristics that alters with the development of desiccation tolerance in recalcitrant seeds is the absence of the deposition of insoluble proteins within vacuoles, which provides mechanical resistance against cell collapse. Accumulated starch and fat stores offer additional volume buffering capability. The development of desiccation resistance depends heavily on the LEAs (late embryogenesis abundant proteins). Different categories of the LEAs have been identified based on certain peptide sequences. The LEAs are tiny, hydrophilic, heat-stable molecules that are unstructured in solution. We still don't know the precise processes by which LEAs guard conventional seeds against desiccation. Dedifferentiation of organelles, metabolic "shut off," and a reduction in endomembrane system components all work together to limit uncontrolled metabolism and the membrane targets of free radicals and reactive oxygen species (ROS) generated as a result. As water concentrations and potentials fall into "intermediate" regions, the latter would be a particular concern. The existence and activity of anti-oxidants suited to the various falling hydration levels experienced by the cells during dehydration underpin all of these occurrences. All the above-mentioned reasons create a huge strain on the storage and preservation of recalcitrant seeds.

Conservation of Recalcitrant Seeds

The majority of stubborn seeds are large, well-hydrated, and metabolically active. Their size, among other characteristics, generally inhibits quick-drying, which is necessary for viability retention at sufficiently low water contents before exposure to cryogenic temperatures. Cryopreservation of large resistant seeds is extremely challenging because they cannot be chilled quickly enough to achieve liquid nitrogen temperature (Bonner, 1990). Consequently, the preferred cryopreservation explant is an embryo, which reflects the genetic identity of the seeds. The first oxidative stress that develops during the processing and recovery of frozen materials happens as a result of having to separate the embryos/axes from the seeds. Two further major sources of harm associated with cryopreservation are further dehydration and ice crystallisation. The majority of stubborn seeds are large, well-hydrated, and metabolically active. Their size, among other characteristics, generally inhibits quick-drying, which is

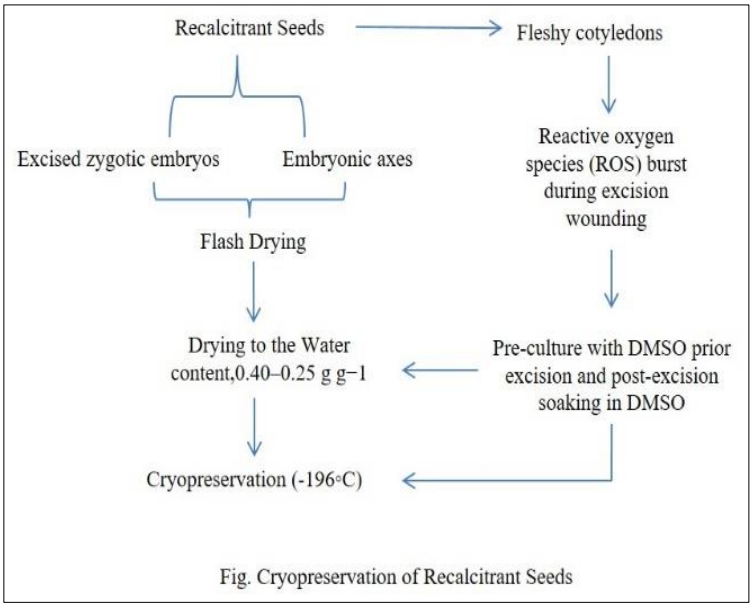

Fig. Cryopreservation of Recalcitrant Seeds

necessary for viability retention at sufficiently low water contents before exposure to cryogenic temperatures. Cryopreservation of large resistant seeds is extremely challenging because they cannot be chilled quickly enough to achieve liquid nitrogen temperature (Bonner, 1990). Consequently, the preferred cryopreservation explant is an embryo, which reflects the genetic identity of the seeds. The first oxidative stress that develops during the processing and recovery of frozen materials happens as a result of having to separate the embryos/axes from the seeds. Two further major sources of harm associated with cryopreservation are further dehydration and ice crystallisation and explants must be partially dried to water content prior to cryogen exposure, limiting (but ideally preventing) ice formation in the tissues. Flash drying embryos /embryonic axis commonly accomplish this (Ballesteros et. al., 2014). Recalcitrant embryos/axes, on the other hand, cannot survive the loss of non-freezable (structure-associated) water and are metabolically active indefinitely; both of these traits are obstacles to successful cryopreservation. Chemical cryoprotectants, such as dimethyl sulfoxide (DMSO), are another option for preventing freezing damage in such seeds (Naidoo et al., 2011). CeIl dehydration is accomplished before freezing in vitrification-based techniques by exposing samples to a concentrated cryoprotective medium and/or air desiccation. Then comes a period of fast cooling. As a result, all elements that affect the production of intracel1ular ice are avoided.

Conclusion

Cryopreservation techniques have advanced to the point where they can be used for immediate use for large-scale experiments in gene banks, despite the fact that desiccation tolerance is not just desiccation tolerance in the case of recalcitrant seeds and that there is a time component to tolerance in the dry state, as a result of research on seeds, particularly seed storage for germplasm conservation. Dehydration sensitivity implies that something that normally confers tolerance in the sensitive tissue is missing, but this has not yet been determined. In actuality, rather than a single process, it is much more likely that a variety of

alternative processes are lacking or malfunctioning. Damage to desiccation-sensitive tissue occurs on various levels, including mechanical damage as a result of volume loss; metabolism-induced ROS production as a result of water removal at intermediate hydration levels. Sensitive tissue cannot endure these potential shocks, whereas tolerant tissue may. Desiccation sensitivity, in practice, limits long-term germplasm conservation due to the inability to store seeds at low temperatures and water levels. Partially drying and quick chilling (and subsequent warming) techniques are used to reduce oxidative stress during drying and to induce cryogenic preservation.

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