Desiccation tolerance mechanisms in seeds: A review

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ABSTRACT

Tolerance to seed desiccation has been reported in seeds of plants grown in dry environments, while sensitivity to dehydration is more commonly found in species that evolved in humid environments (ANGELOVICI et al., 2010). From an evolutionary point of view, the transition from sensitivity to resistance to desiccation marks an important phase in the conquest of land by plants (FRANCHI et al., 2011; SMOLIKOVA et al., 2021).

Keywords: Orthodox seeds, LEA proteins, Antioxidative enzymes.

1 INTRODUCTION

Tolerance to seed desiccation has been reported in seeds of plants grown in dry environments, while sensitivity to dehydration is more commonly found in species that evolved in humid environments (ANGELOVICI et al., 2010). From an evolutionary point of view, the transition from sensitivity to resistance to desiccation marks an important phase in the conquest of land by plants (FRANCHI et al., 2011; SMOLIKOVA et al., 2021).

Seed tolerance to desiccation can be defined as the ability to recover biological functions after dehydration, and this maximum dehydration is a critical point characteristic of each species (BLACK et al., 2002; OLIVER et al., 2020). The ability to tolerate desiccation is acquired soon after the first phase of maturation, even before the natural drying process begins, and seems to be initiated by maternal factors, rather than directly through environmental signals (BEWLEY et al., 2013).

The mechanisms of desiccation tolerance in seeds are strongly connected to programs to regulate their own maturation (ANGELOVICI et al., 2010). The more protective mechanisms available to the cell during the dehydration process, the greater the integrity of the genetic information and the lower the demand for DNA transcripts for the "de novo" synthesis of membrane, organelle and cytoskeletal constituents (MASETTO et al., 2008).

The longevity and viability of seeds that do not have desiccation tolerance mechanisms are very short. On the other hand, seeds that present these mechanisms can be stored for a long period without losing viability (KIJAK; RATAJCZAK, 2020).

In this way, desiccation tolerance is associated with an adaptation strategy that allows the survival of the seed during storage, ensures the development of the plant only when the conditions of the environment are favorable, allowing the dissemination and perpetuation of the species (OLIVER et al., 2020).

Several studies have been carried out to understand the mechanisms that lead to the natural tolerance to drying that most species present. However, not all of them have yet been very well elucidated. Thus, this work aims to compile the published information on this subject, starting with the understanding of seed development, physiological behavior, characterization of parameters associated with maturation, and then understanding the mechanisms associated with desiccation tolerance in seeds.

2 SEED DEVELOPMENT

2.1 PHYSIOLOGICAL BEHAVIOR OF SEEDS

The physiological behavior of seeds was initially studied by Roberts et al. (1973), who classified seeds as orthodox or recalcitrant, according to the type of behavior that the seeds exhibited during development and storage. Later, in 1990, Ellis et al. introduced the concept of intermediates to those seeds that exhibited intermediate behavior between orthodox and recalcitrant.

Orthodox seeds are those that can be dried at levels of less than 7% water and tolerate storage at low temperatures (KIJAK; RATAJCZAK, 2020). They are known as desiccation tolerant because they tolerate the immediate effects of severe water loss (WALTERS et al., 2000). There are reports that these species depend on the reduction in the amount of water in their tissues to redirect their metabolism towards germination (MARCOS-FILHO, 2015).

On the other hand, recalcitrant seeds do not have tolerance to desiccation and drying damage can lead to total loss of viability (MARCOS-FILHO, 2015). In addition to this fact, recalcitrant seeds do not withstand storage at very low temperatures, and may lose viability, depending on the species, at temperatures of 10 to 15 °C, not tolerating freezing (MARCOS-FILHO, 2015). Thus, the longevity of recalcitrant seeds, even under favorable storage conditions, is still quite short. For this reason, the post-harvest conservation of these seeds continues to be an obstacle to agricultural production.

Regarding the physiological changes that occur during maturation, orthodox and recalcitrant seeds present distinct patterns, mainly evidenced in the final stage of development. At the end of seed maturation, two types of behaviors can be observed regarding the water content of the seeds. In orthodox, there is a rapid reduction in water content, leading to a reduction in cellular metabolism and the state of seed quiescence. Metabolism is only reactivated when resources favorable to embryo development are available (BEWLEY et al., 2013).

In recalcitrant seeds, there is no drying process at the end of the seed maturation process. They have a high water content when they detach from the mother plant and die when their moisture content is reduced to values below their critical level. Once the seeds at this stage are fully formed, the germination process begins, which sometimes occurs in the mother plant (BARBEDO; MARCOS FILHO, 1998).

This difference between orthodox and recalcitrant, evident at the end of the maturation process, occurs mainly due to the presence of several mechanisms of desiccation tolerance existing in orthodox and that are not present or efficiently active in recalcitrant women.

Desiccation tolerance mechanisms in orthodox plants are acquired during the process of seed development and maturation.

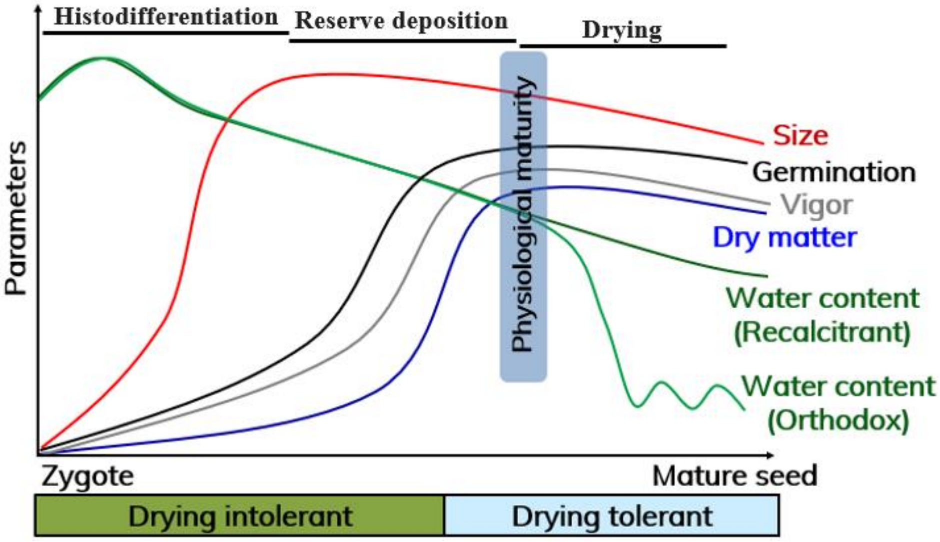
2.2 SEED DEVELOPMENT AND ACQUISITION OF DESICCATION TOLERANCE MECHANISMS IN ORTHODOX

The seed maturation process can be conventionally divided into three phases: histodifferentiation; cell expansion and deposition of reserves; and drying (Figure 1).

The first phase is characterized by high water content, where there is rapid initial growth due to intense cell divisions. This phase is known as the histodifferentiation phase, because it is at this stage that the embryo tissues are differentiating (CASTRO et al., 2004). After this initial growth, the second phase occurs, with expansion, increase in cell size and deposition of reserves, which will be used during the germination process so that the embryo obtains the necessary energy to resume its growth. As the maturation progresses, there is a gradual accumulation of reserves. Simple sugars, such as monosaccharides, become complex, forming oligo- and polysaccharides, which can be stored as starch, cellulose or hemicellulose, as well as reserves of other natures, such as oils, proteins and other compounds, and the chemical composition of the seeds varies according to the genotype, cultivar and production environment (BEWLEY et al., 2013).

While the accumulation of reserves increases, the water content decreases, because, in addition to having a lower demand for water, after cell proliferation, the compounds accumulate and occupy the spaces of the cells previously filled by water. When the seed reaches maximum dry matter, it is said to have reached the point of physiological maturity. From this point, it triggers drying, where the seeds go through a pre-programmed period of reduction in their water content (CASTRO et al, 2004). At post-maturity, drying too quickly can lead to seed collapse. Thus, this period of slow dehydration, with the seeds still in the mother plant, is of great importance in the formation of more tolerant seeds. This gradual desiccation allows the natural development of the seed and protein formation metabolism to resist the consequences of dehydration (MARCOS-FILHO, 2015). Thus, this step is essential to allow the action of protective mechanisms that confer greater tolerance to cellular imbalances caused by dehydration.

Figure 1. Seed maturation process. Development from zygote to mature seed, identifying the modifications of the main technological parameters associated with the maturation phases.



It is also important to emphasize that when dehydration occurs, the cytoplasm condenses and intracellular components become more crowded, providing an environment susceptible to several undesirable interactions, which can result in protein aggregation and denaturation and even organelle fusion (MANFRE et al., 2009). Thus, desiccation tolerance mechanisms emerge as a necessity so that seeds can develop normally.

The seed begins to tolerate dehydration when most of the reserves have already been deposited, corresponding to the beginning of the reduction of abscissic acid (ABA) levels (KIJAK; RATAJCZAK, 2020). The main regulators of maturation and desiccation tolerance are abscisic acid and DOG1 protein, which controls the transcription factor network, represented by LEC1, LEC2, FUS3, ABI3, ABI5, AGL67, PLATZ1, PLATZ2 (SMOLIKOVA et al., 2021). This network is complemented by epigenetic regulation of gene expression through DNA methylation, post-translational histone modifications, and chromatin remodeling. In this situation, the synthesis of reserve proteins ceases and the formation of enzymes and the synthesis of proteins associated with germination begins (SMOLIKOVA et al., 2021).

These fine regulatory mechanisms allow orthodox seeds to maintain desiccation tolerance throughout the germination period up to the radicle protrusion stage. This moment, when seeds lose tolerance to desiccation, is critical for the entire seed development process (SMOLIKOVA et al., 2021).

Premature desiccation affects the synthesis of proteins as well as enzymes essential to development and germination. In this way, the removal of the seeds from the plant and their rapid drying can determine the complete loss of viability, even in orthodox plants (MARCOS-FILHO, 2015).

There is evidence that drying during maturation is also conditioned to a preparation for germination, characterized by an inversion pattern in the metabolic process. The seed gradually reduces synthesis activities and prepares to germinate (MARCOS- FILHO, 2015). This process of seed desiccation to prepare for germination is characterized by several alterations in transcription, post-transcriptional, and metabolic processes. Some of the transcription and metabolic processes associated with germination already begin during seed desiccation, while others are initiated during germination (ANGELOVICI et al., 2010).

During post-maturation, the seed maintains a low level of metabolic activity, which preserves its viability. In addition, during this period, there is a reduction in germination inhibitors, membrane alterations and protein degradation that improves germination vigor (ANGELOVICI et al., 2010).

Seed desiccation is probably regulated by hormonal balance play, sugar signaling and chromatin remodeling (ANGELOVICI et al., 2010).

The acquisition of desiccation tolerance in orthodox seeds is associated with several cellular processes. The main mechanisms include the accumulation of disaccharides and oligosaccharides, final embryogenesis with abundant LEA proteins, heat shock proteins, the activation of antioxidant defenses, changes in the physical structure of the cell, and a gradual and continuous increase in the density of substances (ANGELOVICI et al., 2010).

It has also been suggested that the variability in desiccation tolerance between different plant species is attributed to the physical structure of the internal seed matrix (vitrification), which is apparently involved in the interactions between protein and sugar, organic acid and amino acid complexes (ANGELOVICI et al., 2010). In addition, Wang et al. (2012) also emphasizes the importance of mitochondria in the desiccation/rehydration process and the role of Ca2+ in the structure and function of mitochondrial membranes to tolerate desiccation. Thus, the ability to recover the integrity of mitochondria and respiration is extremely important for all other desiccation tolerance mechanisms to be activated.

LEA proteins are hydrophilic, highly stable, and do not denature by heating. These proteins have different modes of action, but always with a view to protecting against desiccation effects. There is evidence that abscisic acid (ABA) is involved in the effects of water stress in plant tissues, such as the expression of LEA genes (GUIMARÃES et al., 2006; SMOLIKOVA et al., 2021).

Heat shock proteins help stabilize protein conformation, favoring desiccation tolerance in orthodox seeds. In these seeds, an increase in sucrose, raffinose and stachyose is also observed at the beginning of the desiccation process (MARCOS-FILHO, 2005). Non-reducing sugars, such as sucrose and polysaccharides, are thought to be less reactive, and therefore protective components of the lipid bilayer of membranes. It is suggested that sucrose acts in the protection of the structure and functioning of phospholipids, while the oligosaccharides of the raffinose series and stachyose, in addition to serving as reserve material or vitreous state formers, also play a role in the protection of membranes and proteins, placing themselves as substitutes for the water withdrawn in desiccation (GUIMARÃES et al., 2006).

However, it is not in all stages that oligosaccharides are active, protecting the seeds from desiccation. Leduc et al. (2012) found a low proportion of oligosaccharides in mature C. *echinata* seeds and an increase in soluble carbohydrate content by immersing immature seeds (35 and 45 DAA) in PEG solution. However, only the seeds in 45 days after anthesis were able to maintain a high percentage of germination after drying.

Oligosaccharides protect membranes from collapse because they allow the formation of the vitreous state and return to the gel state when rehydrated. The formation of this glassy state in a stable form and the marked interaction of these sugars with water are crucial features of desiccation tolerance (BEWLEY et al., 2013).

During drying, cell damage can be caused by lipid peroxidation. These can be reduced or prevented by protective mechanisms, involving enzymes that scavenge free radicals and peroxides, such as superoxides dismutase (SOD), catalase (CAT) and peroxidase (PO) (GUIMARÃES, 2006). SODs are a group of metalloenzymes that catalyze the disproportionation of free superoxide radicals ($O2^{-}$). They are present in the cell cytoplasm and mitochondrial matrix. It catalyzes the dismutation reaction from $O2^{-}$ to O2 and H2O2, where H2O2 is also decomposed by catalase or peroxidase. This protection can also be carried out by antioxidant molecules present in the seeds, such as ticiferius, B-carotenes or ascorbic acid. Tocopherols and B-carotenes block lipid peroxidation and ascorbic acid acts on hydrophilic tissues (GUIMARÃES, 2006).

Other desiccation protection mechanisms can also be directed to the cytoplasm, where it protects against the crystallization of solutes and proteins. This protection is given through vitrification, that is, the formation of a high-viscosity liquid substance that does not form crystals and is stable over a wide temperature range. This viscosity decreases the occurrence of chemical reactions, keeping the pH of the medium stable, preventing freezing and promoting tolerance to extreme temperatures and avoiding the collapse of cells with desiccation (GUIMARÃES et al., 2006).

Thus, desiccation tolerance seems to be measured by protection systems that prevent lethal damage to different cellular components, including membranes, proteins, and cytoplasm. There is an accumulation of reduced sugars that stabilize membranes and proteins in dry conditions and promote the formation of the vitric phase in the cytoplasm. There is the ability to prevent, tolerate or repair a free radical attack during desiccation and also an efficient defense through the LEA proteins that are induced by ABA.

The mechanisms behind desiccation tolerance are activated at the late stage of seed maturation and are associated with the accumulation of proteins abundant in late embryogenesis (LEA), small heat shock proteins (sHSP), non-reducing oligosaccharides, and antioxidants of different chemical natures (SMOLIKOVA et al., 2021).

It is true that all components of desiccation tolerance are extremely important and that tolerance is acquired through these mechanisms acting together and in synergy.

3 FINAL THOUGHTS

Desiccation tolerance in seeds is extremely important for the longevity, dissemination and perpetuation of plant species. In agricultural production, it allows the reduction of water content at the end of the maturation process, which culminates in metabolic rest, preserving the quality of the seeds in storage.

There are several tolerance mechanisms that allow the dehydration and rehydration of seed tissues without cell collapse. Among these, the action of abscissic acid and DOG1 protein, LEA and heat shock proteins, the action of oligosaccharides and the vitric state, and the antioxidative defense complex of seeds stand out. In addition, there is epigenetic regulation of gene expression through DNA methylation, post-translational modifications of histones, and chromatin remodeling.

REFERENCES

ANGELOVICI, R.; Galili, G.; FERNIE, A. R.; FAIT, A. Seed desiccation: a bridge between maturation and germination. Trends in Plant Science, v. 15, n. 4, p. 211-218, 2010.

BARBEDO, C.J. ; MARCOS FILHO, J.. Tolerância à dessecação em sementes. Acta Botanica Brasilica, v. 12, n.2, p. 145-164, 1998.

BEWLEY, J. D., BRADFORD, K. J.; HILHORSTE H. W. M.; NONOGAMI, H. Seeds: physiology of development, germination and dormancy 3.ed. New York: Springer, 2013, 406p.

BLACK, M.; OBENDORF, R. L.; PRITCHARD, H. W. Damage and tolerance in retrospect and prospect. In: BLACK, M.; PRITCHARD, H. W. (Ed.). Desication and survival in plants: drying without dying. Wallingford: CABI, 2002. p. 367-382.

CASTRO, R. D.; BRADFORD, K. J.; HILHORST, H. W. M. Desenvolvimento de sementes e conteúdo de água. In: FERREIRA, A. G.; BORGHETTI, F. (Orgs.) Germinação: do básico ao aplicado. Porto Alegre: Artmed, 2004. p.51-68.

ELLIS, R. H.; HONG, T. D.; ROBERTS, E. H. An intermediate category of seed storage behaviour? I. Coffee. Journal Experimental Botany, Oxford, v. 41, n. 230, p. 1167-1174, 1990.

FRANCHI, G. G.; PIOTTTO, B.; NEPI, M.; BASKIN, C. C.; BASKIN, J. M.; PACINI, E. Pollen and seed desiccation tolerance in relation to degree of developmental arrest, dispersal, and survival. Journal of Experimental Botany, p. 1-15, 2011.

GUIMARÃES, R. M.; OLIVEIRA, J. A.; VIEIRA, A. R. Aspectos fisiológicos de sementes. Informe Agropecuário, v. 27, n. 232, p. 40-50, 2006.

KIJAK, H.; RATAJCZAK, E. What Do We Know About the Genetic Basis of Seed Desiccation Tolerance and Longevity? International Journal of Molecular Sciences, v. 21, e3612, 2020.

LEDUC, S. N. M; SILVA, J. P. N; GASPAR,M; BARBEDO, C. J.; RIBEIRO, C. L. F. Non-structural carbohydrates of immature seeds of Caesalpinia echinata (Leguminosae) are involved in the induction of desiccation tolerance. Australian Journal of Botany, v. 60, n. 1, p. 42-48, 2012.

MANFRE , A.J. , LAHATTE , G.A. , CLIMER , C.R. E MARCOTTE , W.R. , Jr. Seed dehydration and the establishment of desiccation tolerance during seed maturation is altered in the Arabidopsis thaliana mutant atem6 -1. Plant Cell Physiology, v. 50, n.2, p. 243 –253, 2009

MARCOS-FILHO, J. M. F. Fisiologia de sementes de plantas cultivadas. Londrina: Abrates, 2015. 660p.

MASETTO, T. E.; FARIA, J. M. R.; DAVIDE, A. C.; SILVA, E. A. A. da. Desiccation tolerance and DNA integrity in *Eugenia pleurantha* O. Berg. (myrtaceae) seeds. Revista Brasileira de Sementes, v. 30, n. 2, p.51-56, 2008.

OLIVER, M. J; FARRANT, J. M; HILHORST, H. W. M.; MUNDREE, S.; WILLIAMS, B.; BEWLEY, J. D. Desiccation Tolerance: Avoiding Cellular Damage During Drying and Rehydration. Annual Review of Plant Biology, v. 71, n.1, p. 435 -460, 2020.

ROBERTS, E. H. Predicting the storage life of seeds. Seed Science and Technology, v. 12, p. 499-514, 1973.

SMOLIKOVA, G.; LEONOVA, T.; VASHURINA, N.; FROLOV, A.; MEDVEDEV, S. Desiccation Tolerance as the Basis of Long-Term Seed Viability. International Journal of Molecular Sciences, v. 22, n. 1, p. 1 - 24, 2021.

WALTERS, C. Levels of recalcitrance in seeds. Revista Brasileira de Fisiologia Vegetal, v.12, p. 7-21, 2000.

WANG, W-Q.; CHENGA, H-Y; MØLLERA, I. M.; SONG, S-Q. The role of recovery of mitochondrial structure and function in desiccation tolerance of pea seeds. Physiologia Plantarum, v. 144, n. 1, p. 20-34, 2012.