333

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Review Article

Resurrection plants: Imperative resources in developing strategies to drought and desiccation pressure

Afroz Alam[1]*, Ananya Dwivedi[1] & Iwuala Emmanuel[2]

[1]Department of Bioscience and Biotechnology, Banasthali Vidyapith, India
[2]Department of Plant Science, Federal University OyeEkiti, Nigeria

Abstract

Resurrection plants are the vital assets of nature that have amazing mechanism to restrict the negative impacts of drought or desiccation stress by diminishing cell damage. These surprising plants are in minority on this planet but have the potential to serve as a powerful resource for developing new strategies for major crop plants that are unable to adapt well to the arid climate. In this review, an attempt is made to highlight the potential aspects of these resurrection plants especially the genetic engineering facet which has been done to develop drought tolerance in economically important plants.

Keywords: resurrection; poikilohydry; stress; tolerance.

*Correspondence
Afroz Alam
✉ afrozalamsafvi@gmail.com

Introduction

Stress is an "undeniable pressure of some antagonistic force", that leads to the alteration or inhibition in the typical functioning of the system. Drought is considered as one of the most important stresses imposed by the environment that delimits the growth and development of plant. Consequently, impairs performance and modulates the production of cash crops more than any other factor (1). Drought stress is either encountered by the plant when there is limitation in water supply to the roots or when the transpiration rate becomes very high. To neutralize severe damages caused by drought, plants employ a range of metabolic adjustment to preserve regular functioning.

Drought tolerance is the level at which there is an adaptation in the arid and water deficit conditions. This mechanism is attributed by osmotic adjustment which involves inorganic ions, organic acids and carbohydrates and the alteration in the elasticity of cells and tissues. Desiccation tolerance is the property of plants to survive in less or almost nil cellular water level and to revive all lost protoplasmic water upon rehydration (2). Resurrection plants are those plants which have the ability to produce pollen or seeds which are tolerant to desiccation and possess vegetative tissues that can tolerate desiccation. They are widely distributed in different taxa varying from pteridophytes to dicotyledons except for the

Table 1. List of some widely studied resurrection plants (14)

Sl. No	Name	Family	Monocot/ Dicot/others	Origin	Poikilochloro phyllous (P) / Homoiochloro phyllous (H)
1	*Xerophyta viscosa* Baker	Velloziaceae	Monocot	Southern Africa	P
2	*Xerophyta humulis* (Baker) T. Durand & Schinz	Velloziaceae	Monocot	Southern Africa	P
3	*Mysothamnus flabellifolius* Welw.	Myrothamnaceae	Dicot	Southern Africa	H
4	*Sporobolus stapfianus* Gand.	Poaceae	Monocot	Southern Africa	
5	*Eragrostis nindensis* Ficalho & Hiern	Poaceae	Monocot	Southern Africa	
6	*Craterostigma plantagineum* Hochst.	Scrophulariaceae	Dicot	Southern Africa	H
7	*Craterostigma wilmsii* Engl.	Scrophulariceae	Dicot	Southern Africa	H
8	*Lindernia brevidens* Skan	Linderniaceae	Dicot	East Africa	H
9	*Boea hygrometrica* (Bunge) R. Br.	Gesneriaceae	Dicot	China	H
10	*Bornetella nitida* Munier-Chalmas ex Sonder	Dasycladaceae	Dicot	Africa, Australia, Asia	H
11	*Selaginella lepidophylla* (Hook. & Grev.) Spring	Selaginellaceae	Pteridophyta	North and South America	
12	*Tortula ruralis* (Hedw.) Gaertn., Meyer & Scherb.	Pottiaceae	Bryophyta	North America	

gymnosperms (3,4). Farrant and Moore (2011) quoted "genes that ensure desiccation tolerance are present in all plants, but are utilized mostly when they produce seeds (5). On the other hand resurrection plants are able to switch on these genes in their leaves and roots whenever there is drought stress". Tolerance against desiccation in resurrection plants was acquired at the latter stages of organismal complexity through the evolution of various metabolic and structural protective mechanisms. These are because either of the combination of ubiquitous housekeeping mechanisms or by the invention of genes that are specific for a particular species like CDT-1 gene in *Craterostigma plantagineum* (6-8).

Places which observe scanty and occasional rainfall are the natives for resurrection plants. Under these adversities they can tolerate by being dry for a wide temperature ranges, where they experience repeated cycle of drying and dehydration all around the year (8, 9). Southern hemisphere region of Africa, Australia, India and South America are the places where most resurrection plants have been reported (10). Initially in 1970s most of the resurrection plants were described (3, 11-14). Resurrection plants are found both in the monocot and dicot plants. Dicot families such as Linderniaceae, Scrophulariaceae, and Myrothamnaceae show the presence of resurrection plants while they are more scattered among the monocot families (Table 1). Genera *viz.*, *Craterostigma* and *Lindernia* represents desiccation-tolerant plants which was suggested by the first phylogenetic analysis among the Scrophulariaceae (15).

Strategies in resurrection plants against desiccation

Morphological changes upon desiccation

Morphology is greatly affected during water deficit conditions in resurrection plants. Leaf curling and folding is the most obvious response during desiccation (16-20). UV radiation causes damage by oxidative stress which can be limited by leaf folding hence it is an important adaptation upon desiccation. *Craterostigma wilmsii* or *C. plantagineum* leaves, are curled inwards during stress. They become tightly folded in such a manner that the abaxial surfaces of mature leaves present in the outer whorl exposed to direct sunlight (Fig. 1) (21, 22). Flat and grass like leaves are observed in *X. humilis*. In desiccated conditions, the folding in the leaf blades are half along the midrib and only the abaxial surface is exposed to direct sunlight (21, 23).

Membrane flexibility and fluidity

Inward shrinking of the central vacuole due to the loss of water causes withdrawal of the cellular contents which leads to a reduction in elasticity between plasmalemma and cell wall. These are lethal when the plasmodesmatal connections are broken (24). Resurrection plants are adapted to protoplast shrinkage undergoes extensive folding in its cell wall, this leads to the constriction of the cell and prevents the plasmalemmato tear from the cell wall (22, 25-28). This leads to the reduction for the capacity of irreversible mechanical damage phenomenon and avoids the negative turgor pressure to develop (29). In the cell wall of *C. wilmsii*, there is a significant level of increment in un-esterified pectins and xyloglucans during

desiccation (27). In *Boea hygrometrica* cell wall flexibility has been correlated with the increase in a glycine-rich protein (30). Membrane fluidity is the result of high degree of poly unsaturation in membrane phospholipids (31).

Photosynthetic capacity of resurrection plants

Resurrection plants modulate their photosynthetic machinery during dehydration either by shutting it completely or operating it in a passive mode to avoid the accumulation of reactive oxygen species (ROS). In *Boea hygrometrica* and *Haberlea rhodopensis* ratio of pigment–protein complexes and the integrity of thylakoids were maintained while there were changes in carbon fixation and disruption in the functions of photosystem II (PSII) (32-33). The decrease of CO_2 assimilation due to the closure of stomata down regulates the activity of PSII. Non photochemical quenching is triggered in leaves of *Ramonda serbica* to control the formation of ROS and to protect the integrity of membrane (34). Dehydration affects the occupation of the enzymes involved in Calvin cycle more pronouncedly than the enzymes involved in electron transport chain (35).

Homoiochlorophyllous species and Poikilo-chlorophyllous species: Two schemes undertaken by resurrection plants to combat oxidative damage

Homoiochlorophyllous species are able to employ the chlorophyll and the thylakoid membranes, while a slight change in the distribution of photosynthetic pigments is being observed (36).

Chloroplasts are modulated during dehydration. They become orbicular with alteration in inner membranes and stacking. The ratio of lipids and proteins is also altered (22, 26, 37-38). In poikilochlorophyllous species, the photosystem complexes and chlorophyll are broken down (T). Accumulation of toxic reactive oxygen species is avoided by the decadence of chlorophyll. Poikilochlorophyllous plants are slower in resuming photosynthesis than homoiochlorophyllous plants as they synthesize all cellular components over again. In comparison with Poikilochlorophyllous plants, Homoiochlorophyllous plants require an effective antioxidant protection in opposition to free radicals (33). HDT (homoiochlorophyllous desiccation-tolerant) plants minimize the production of reactive oxygen species not only by the folding of leaves to prevent the interaction between light and chlorophyll but also by alternative mechanisms as well.

The first mechanism is the scavenging of reactive oxygen species through antioxidant enzymes and metabolites which helps to protect the plant during desiccation (18). Second mechanism is the accumulation of xanthophylls or anthocyanins which ultimately minimizes ROS formation (21, 22).

The final two mechanisms through which homoiochlorophyllous plants reduce ROS development include downregulating the formation of gene products, which is related with photosynthesis, such as Chlorophyll a/b-binding

Fig. 1. Variation in the morphology of resurrection plants *Xerophyta humilis* (a and b) and *Craterostigma plantigenum* (c and d).

protein, RuBisCO large subunit (rbcl), or oxygen-evolving complex (40) and the initiation of shielding proteins such as early light-inducible proteins (36, 41). Thylakoid chlorophyll binding proteins (ELIPs) are being demonstrated transiently during the de etiolation of etiolated plants (42). Mature leaves, when exposed to excessive sunlight and other abiotic stresses start the formation of ELIPs, which consequently leads to the amplification of ROS (43-47). In the leaves of *C. plantagineum* an unusual gene family has been indicated in response to desiccation (48). The genes that encode plastid-targeted proteins (CpPTP), are capable to negotiate with plastid DNA and protect it against dehydration.

Antioxidant enzymes protection

Establishment of numerous antioxidant protective mechanism is correlated with recovery in resurrection plants during desiccation and preserve them upon rehydration (49, 50). High concentrations of chlorophyll is retained in *M. flabellifolius* during desiccation. When it is rehydrated, antioxidants such as α-tocopherol, ascorbate and glutathione are accumulated in different tissues. In the recovery pathway, antioxidants are an essential component. It is observed in plants, which are unable to recover from desiccation because antioxidants are broken down due to long exposures of light (49). A novel antioxidant enzyme induced by desiccation corresponding to a type of 1-cys peroxiredoxin (Prxs) was identified in the leaves of *X. viscosa* (51-52). In substrates such as hydroperoxides they are found to be active (53).

Abscisic acid role in desiccation tolerance pathways

Water deficiency induces the stomata to close and scope for the assimilation of carbon is reduced, which in turn results in the non-availability of electron acceptor (NADP) and consequently leads to the formation of free radicals (54-58). Abscisic acid (ABA), is the phytohormone that aids in the regulation of the pore size of stomata and regulates the inlet of CO_2 and the outlet of water from the leaf. By the action of ABA pores of stomata close in response to drought stress, this leads to an increment in cytosolic calcium concentrations via Hydrogen peroxide (H_2O_2) activated channels and release from the vacuole and other intracellular stores (58-59). Particularly H_2O_2 is thought to have a role in signalling in guard cells , stimulates the anion channels in the plasma membrane, ending into guard cell depolarization, efflux of potassium ions and depletion of turgidity and volume, concluding in the termination of stomata (60). This also leads to the activation of ROS in guard cells, enabling the stomata to shut down. ABA is an important hormone which is useful in attaining tolerance against desiccation and also stimulates the synthesis of desiccation induced proteins in most of the resurrection plants including the aquatic species *Chamaegigas intrepidus* (16, 61-66).

Carbohydrate metabolism

It is commonly pragmatic that the accumulation of soluble sugars during the desiccation process has is significance to provide tolerance during stress. Glucose is formed rapidly from starch during desiccation (67). Functions of sugars include osmotic adjustments during dehydration and stabilization of proteins in the dehydrated cells (68). Protein denaturation and membrane fusions are prevented by sucrose and trehalose (67, 69). They have the capacity to form bioglasses (vitrification) within the dried cell. Sugars are not solemnly responsible for the glass formation in the cytoplasm. It may be due to the interactions between sugars and supplementary molecules, probably with proteins (70). The production of free radicals is limited and slowed by chemical revulsion and molecular dissipation in cytoplasm in the process of vitrification (70). Hence, it is evident that vitrification holds an important place in the protection of resurrection plants. Sucrose level exceeds in dry leaves and becomes a potent sugar during dehydrated condition in *S. stapfianus* while in the hydrated state glucose, fructose, and galactose are present in large amounts (71). An eight-carbon sugar 2-octulose, is predominantly found in hydrated leaves of *C. plantagineum*, which is transformed to sucrose upon rehydration (72). While, stachyose is accumulated in the roots of drying plant. Sucrose biosynthetic genes are induced in *C. plantagineum* by both desiccation and abscisic acid (73). Transketolase contributes to the conversion of octulose to sucrose.

Proteins having protective properties

At the molecular level, a marked build up of stress related proteins is the most obvious change during dehydration. Majority of these are Late Embryogenesis Abundant (LEA) proteins. The ability to uphold the usual metabolism without any irreversible damage during extreme scarcity of cellular water level is known as desiccation tolerance (2). There is principally high levels of LEA protein mRNAs exist in the dehydrated mature embryos, whereas, during late embryogenesis stage, the transcripts of storage protein genes are completely degraded. These proteins operate as molecular chaperones and play dominant roles in biosynthesis of proteins, folding, assembly, intracellular localization, secretion, and degradation of other proteins (74). They also act as stabilizers, hydration buffers, antioxidants, membrane protectants, organic glass formers and ion chelators (75).

Besides LEA proteins, heat shock proteins (Hsps) are also linked with desiccation. Usually, Hsps have the ability to maintain protein partners in a folded or unfolded nature, reduces the accumulation of external proteins and are

removed from the cell as they tend to disturb its proper functioning (74).

Rehydration

The mechanism of rehydration was in some ways have been overlooked in the discovery of resurrection plants. Rehydration involves a slow retrieval of cellular kinetics, which ultimately leads to the reactivation of normal metabolic functions. The entire desiccation tolerance is based on the reestablishment of metabolic activities and important functions.

A discrete period of time is required for the successful rehydration in *C. plantagineum* (63). A complete uptake of water for 12–15 h is required by dried *Craterostigma* plants for its rehydration. There were no detection of new mRNAs during the early stages of rehydration from protein assays (76). mRNAs and enzymes which are important for the restoration of respiration, photosynthesis and other metabolic pathways were sufficiently protected in the dehydrated condition. Processes for averting repair mechanism of cell damage upon rehydration were found in resurrection plants (77). Proteins such as EXPANSINs play vital role in rehydration (78). AQUAPORINs are important for regulating the rate of flow of water inside the cells upon rehydration and produce and store mRNAs during the last stage of drying (78).

Signalling mechanisms in resurrection plants

In response to water deficiency, there is an immense transcriptional reprogramming. ABA, lipid messengers or alterations in the redox components are some types of signalling molecules present in resurrection plants (47, 79). Induction of various protective mechanism including inhibition of photosynthesis and retardation of growth are due to the presence of transcription factors from different families (47, 79-81).

In *Boea hygrometrica,* the synthesis of raffinose and raffinose family oligosaccharides (RFOs) in an ABA dependent manner. It is due to transcription factor (WRKY) which attaches to a W-box promoter element of the galactinol synthase gene (30).

In *C. plantagineum* CpMYB10, transcription factor from the Myb family, not only binds to and regulates its own promoter but also the promoter of the late embryogenesis abundant gene (*LEA* gene Cp11-24). It functions as an activator stressed tissue and as a repressor in non-stressed tissues (81). In leaves and roots of *Xerophyta humilis,* vicinaloxygen chelate (VOC) metalloenzyme superfamily are being upregulated during desiccation (82, 83).

Transcriptome profiling in resurrection plants

Transcripts of two dicot resurrection plant species namely *C. plantagineum* and *Haberlia rhodopensis* were sequenced from their different physiological stages. This allowed the coverage of the entire genome of the transcripts in species with genomes which are still not be sequenced. Transcript sequences of these two resurrection plants are similar to genes from castor bean (*Ricinus communis*), poplar (*Populus trichocarpa*) and grapevine (*Vitis vinifera*). Gene ontology analysis identified categories prominently represented in particular conditions. Genes in unstressed samples of both species were related with growth, cell wall organization and photosynthesis. While genes recovered after dehydration were namely sucrosesynthase, chromosome scaffolded proteins. While many proteins related with stress are known, including various LEA proteins, LIPOCALIN, a GABA transaminase and pathogenesis-related (PR) proteins. A range of signalling related genes encoding proteins have been reported that are associated with calcium channeling and ABA signalling in *C. plantagineum* (63). In *H. rhodopensis* LEA proteins, catalase genes, early light inducible proteins (ELIPs), heat shock factors, protein phosphatase genes have been recognized (36, 84).

Cconclusion

In today's scenario where human population is increasing minute by minute which is ultimately leading to damage to the ecosystem intentionally or unintentionally. Drought is one such damage which is a threat to agriculture. Genes from resurrection plants have the potential for molecular improvement in crop species which are subjected to drought and desiccation. Hence, resurrection plants are considered as the model plant for drought tolerance. To engineer desiccation tolerance in different species there is a requirement of a complex approach. Results of the past molecular experiments suggest that there are some novel genes in resurrection species but that almost all products and by-products of gene which are being expressed are correlated to desiccation tolerance and have their substitutes in non-desiccation tolerant plant species. Examples of these novel proteins and metabolites are the hydrophilic protein CpEdi-9 from *C. plantagineum* (85) and 3,4,5-tri-O-galloylquinic acid which is being isolated from *M. flabellifolia* (86). Desiccation tolerance in resurrection plants is due to the combined effect of the collection of antioxidants and different osmo-protective compounds namely sugars (sucrose and raffinose), trehalose, some hydrophilic proteins such as LEA proteins. Poikilochlorophyllous and homiochlorophyllous resurrection plants have different machineries which help them against desiccation. Resurrection plants in expense to extreme stress grow slowly this enables their protection against desiccation and helps in the formation of genes that response to stress. Dehydration/rehydration responses and functional analysis of genes (87) aid us in understanding unique cellular processes of tolerance in resurrection plants which aimed to

serve as the base for improvement in vital crop species at the molecular level.

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Conflict of Interest

Authors declare no conflict of interest.

Authors' contributions

All the authors contributed equally to the work presented in this paper.

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