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Crops for dry environments

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Climate change necessitates increased stress resilience of food crops. We describe four potential solutions, with emphasis on a relatively novel approach aiming at true tolerance of drought rather than improved water-holding capacity of crops, which is a common approach in current breeding and genome editing efforts. Some Angiosperms are known to tolerate loss of 95% of their cellular water, without dying, not dissimilar to seeds. The molecular mechanisms and their regulation underlying this remarkable ability are potentially useful to design tolerant crops. Since most crops produce desiccation tolerant seeds, genomic information for this attribute is present but inactive in vegetative parts of the plant. Based on recent evidence from both seeds and desiccation tolerant Angiosperms we address possible routes to 'flipping the switch' to vegetative desiccation tolerance in major crops.

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Introduction

Severe drought is one of the climatic factors that is most destructive to production of food crops worldwide. Climate models predict an increase in severity and duration of drought in most regions of the world where agriculture is mainly rain fed. Combined heat and drought, together with deforestation and other anthropomorphic causes, have caused increased aridification and desertification and thus diminished the areas of arable land. Since this process is not readily stopped or reversed, it is imperative to develop a climate-smart agriculture to maintain food security for the growing world population in the foreseeable future. Here we will address difficulties and opportunities to design or recruit food crops that survive long-term severe drought.

Scenarios to acquiring drought-resilient crops

Of the several hundred thousand known plant species, some 120 are cultivated for human food. Nine supply over 75% of global plant-derived energy intake and of these, only three – wheat, rice and maize – account for more than 50% thereof. Breeding programs of most of these crops have been selecting for high yields under near-optimal conditions with an inevitable loss of abiotic stress resilience. Further selection for shorter growing seasons, greater root length, and/or improved resistance to water loss under drought conditions have aimed at surviving short dry spells. However, such crops, and indeed most angiosperms, are intolerant of water deficit stress, dying after loss of between 10% and 60% of total cellular water depending on the species [1]. Thus, under extended periods of drought crop loss is inevitable, especially in developing countries where resources for modern agriculture, such as irrigation, are virtually non-existent [2•].

There are various potential routes to acquiring drought-resilient crops. We place more emphasis on the last scenario as this is the most unexplored and presents a case for the most extreme but successful strategy for extending agriculture into extreme environments (Figure 1).

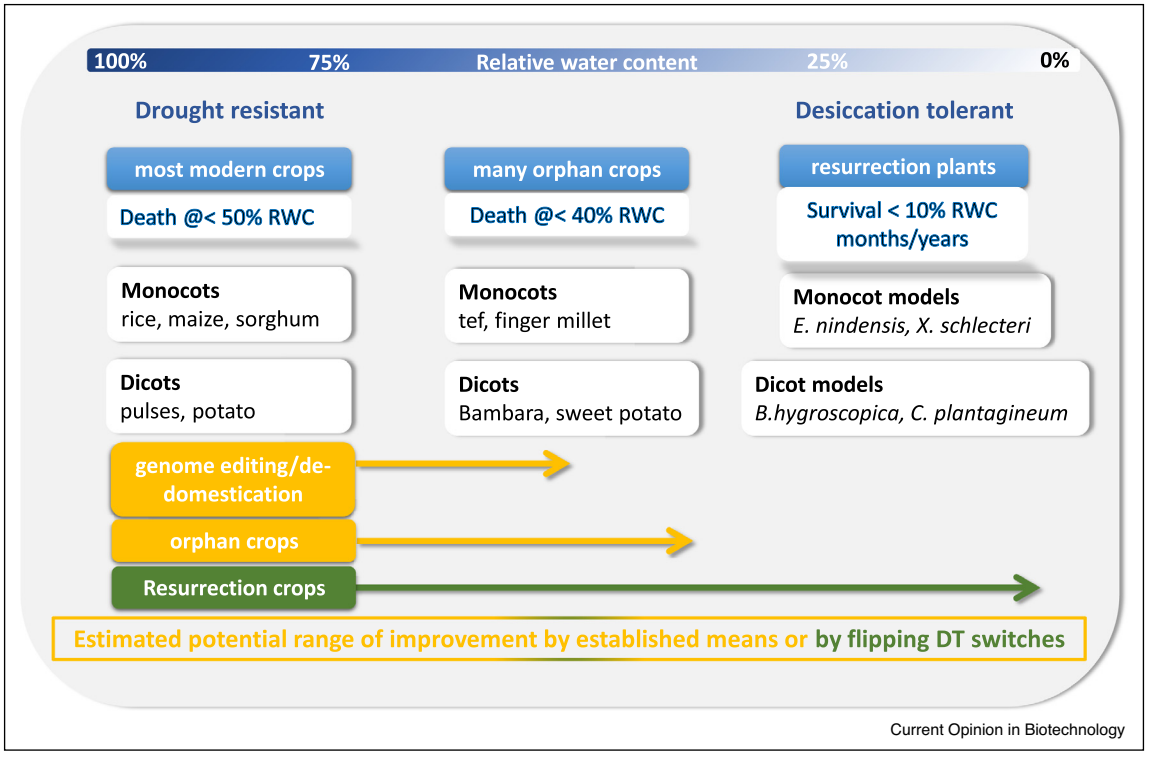
Employing indigenous crops

Indigenous or 'orphan crops' are very often resilient to a plethora of stresses [3]. These crops have long been used as the major staple food crops in many developing countries because of their particular role in nutrition, food security and income generation to resource-poor farmers. Typical examples of orphan crops are Teff (*Eragrostis tef*), this being the main staple in Ethiopia and sweet potato (*Ipomea batatas*), native to central and south America and indigenised in many countries in Africa (Figure 1). Both are considered drought tolerant, the former surviving up to 60% water loss before dying [4•], the latter maintaining hydration longer periods under drought conditions due to its prolific root system [5]. Conforming to the trade-off between yield and stress tolerance, these crops have relatively low yields. However, because of their relative resilience, they may be grown in more marginal areas and thus make up for the loss in yield per hectare.

Genome editing of current crops

Promising technologies such as CRISPR-Cas9 allow very precise modification of genes, and potentially of stress tolerance. Since plants possess substantial phenotypic plasticity, most plant traits are multigenic. Therefore, single-gene modifications to enhance abiotic stress tolerance are unlikely to be effective. Thus, more molecular

Figure 1



Overview of critical water contents limiting current and orphan crop survival and estimation of increase in the range of water deficit tolerance, and thus areas in which crops could be grown, if modified in the ways proposed in this article. Examples of modern crops and orphan crops that could be modified for improved water deficit tolerance, and resurrection plants that could be used as models for induction of vegetative desiccation tolerance are given.

information underlying these traits is required for meaningful modification of multiple genes. Moreover, the inherent trade-off between plant growth and stress tolerance requires additional genome editing, for example, by overexpressing growth-related genes that are suppressed by abiotic stress [6]. An additional complication is that genome editing of crops relies on genetic transformation and plant regeneration which are still a bottleneck in the process, particularly for the 'smaller' crops, such as the orphan crops. Clearly, innovations are needed to enable genome editing in crops at higher efficiency [7]. Because of these caveats not many second generation high-yielding stress-tolerant transgenic crops have been released to the market yet [6].

De-domestication of current crops

De-domestication is an evolutionary process by which domesticated crops reacquire wild-type traits and may form independent reproducing populations [8]. De-domesticated populations may contain important genetic resources for modern crop breeding. Extensive phenotyping of such populations may yield desirable traits including drought and heat tolerance. Detailed genetic analysis of de-domestication pathways has become possible since the introduction of next generation sequencing. De-domestication is a result of natural variation by natural mutations. Mutagenesis of crops and extensive screening for desirable traits is not dissimilar to this and is widely employed. However, both these paths of action will at best produce a phenotype similar to that of an orphan crop.

Using resurrection plants as models to bioengineer desiccation tolerance in crops

Resurrection plants display vegetative desiccation tolerance, being able to tolerate loss of 95% of their cellular water, for prolonged periods, without dying [9•]. Together with seeds, they present the most extreme plant response to drought, and thus are exceptional models for improvement of water deficit tolerance in crops [10]. As desiccation tolerance is a result of coordinated action of multiple signalling cascades, it is unlikely that use of single or small cassettes of genes from a resurrection plant will bring about an extremely drought tolerant phenotype. Indeed attempts at doing so in model organisms such as *Arabidopsis* or tobacco [11–13] or even in African subsistence crops such as sweet potato [14•] and maize [10; unpublished observations] have shown some improvement in

tolerance of short term drought imposed under laboratory conditions, due to improved water retention under those conditions, rather than ability to tolerate more extreme water loss. Field testing of transgenic crops may or may not bring out the desired phenotype obtained in the lab, and field trials attempted (e.g. in the Water Efficient Maize for Africa project) rather show improved water use efficiency than water deficit tolerance. Relative water contents of the crops are hardly ever measured. Thus, the most promising way forward in obtaining a resurrection phenotype is in the use of technologies such as CRISPR-Cas9 to manipulate suites of genes that are likely to be required for desiccation tolerance.

Molecular mechanisms underlying desiccation tolerance

Genome wide studies of resurrection Angiosperms have revealed a striking similarity with seeds in the nature of molecular mechanisms that are recruited to mitigate desiccation damage [15,16•]. The protective components of these mechanisms are found across all lineages of the Plant Kingdom and include late embryogenesis abundant proteins (LEAs), heat shock proteins (HSPs), antioxidants and specific sugars [9•]. Arguably, vegetative desiccation tolerance is an evolutionary rewiring of seed desiccation tolerance that has occurred in several independent events across Angiosperm taxa. Modern and orphan crops produce desiccation tolerant seeds, which implies that the genomic information for desiccation tolerance is contained within vegetative tissues but is suppressed. This opens possibilities for designing crops with extreme tolerance of water deficit by utilizing this 'inherent' desiccation tolerance. Indeed, this could involve activating/deactivating abscisic acid-(ABA) controlled key genetic switches which we postulate variously below. Likely first candidates are ABI3 and its orthologs [15] and ABF or other master regulators [16•].

The activation of such switches in vegetative tissues may be mediated by epigenetic regulation. In seed maturation, the ABA-controlled LAFL (LEAFY COTYLEDON 1 and 2, ABSCISIC INSENSITIVE 3, and FUSCA 3) suite of transcription factors maintain the seeds in a developmental (desiccation tolerant) state [17]. The LAFL genes are subject to extensive chromatin modification [reviewed in Ref. 17]. For example, active ABI3 and LEC2 genes are associated with H3K4me3, whereas their repression is associated with H3K27me3. Thus, elimination of histone acetylation is required to maintain repression. Polycomb Repressive Complexes PRC1 and 2 are pivotal in this process [17]. As the LAFL network is under epigenetic control and, hence, prone to epigenetic priming by the environment [18]. There is ample evidence for priming-induced DT in vegetative tissues. For example, DT can be re-induced by abiotic stress or ABA in protruded radicles of seedlings [19]. Seedlings of *Xerophyta schlecteri* become desiccation tolerant only after a similar treatments [15]. Finally, mild drought acclimation induces rapid desiccation tolerance in the dicot resurrection plant *Boea hygrometrica* [20]. Although in all of these cases the molecular mechanisms remain to be elucidated, an epigenetic activation/deactivation of a LAFL-like network is a plausible model since a permanent loss of function of one or more of these master regulators would also make seeds desiccation sensitive. Thus, a reversible control of a LAFL-like network would be the most versatile way to render desiccation tolerance to leaves, independent of the seeds.

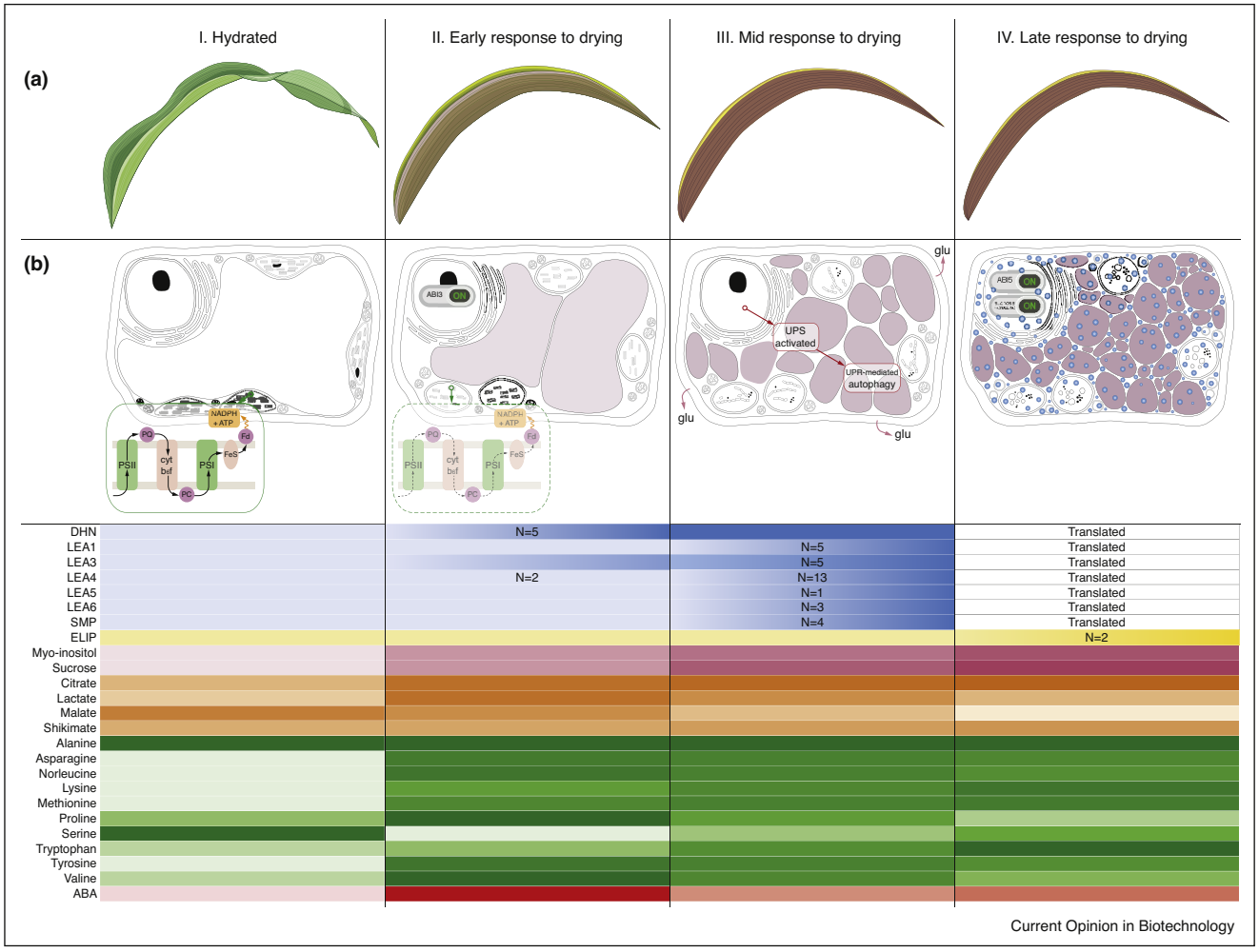
Model RPs for monocots and dicot crops

There is considerable evidence however, that there are inherent differences among resurrection plants with respect to the fine detail of molecular mechanisms employed in desiccation tolerance. Thus for practical purposes it is important to match specific resurrection plants as models for specific crop types. At the broadest level the well-studied monocot *X. schlecteri* (ex *Xerophyta viscosa*) [10,15,21,22,23•,24] can be (and is, see above) utilized as a model for cereal crops such as maize whereas the similarly well studied eudicots like *Craterostigma plantagineum* [25,26,27••] and *B. hygrometrica* [20,28,29•,30] may serve as models for dicots such as legumes.

Using evidence from research conducted on such species we present schematic representations of changes likely to be required in a monocot crop such as maize (Figure 2) and a dicot crop such as soybean (Figure 3) to survive extreme water loss. While there are common features, significant differences are evident as a consequence of leaf and cell morphology, mechanisms of photoprotection employed and choice of carbon and nitrogen stores required for subcellular stabilization in the drying and desiccated states, and for immediate energy on rehydration. A general observation of changes occurring on dehydration of RPs is that leaf tissues appear to go through an early (ERD), mid (MRD) and late (LRD) response to drying in which shifts in metabolism and nature of transcription and translation change. Changes occurring in the ERD, while more typical of those also occurring in crops, are crucial to successful continuance into the MRD and in turn those occurring in this stage are crucial to successful completion of the LRD.

During the ERD, in which *ca* 40% of cellular water is lost, turgor is maintained by presence of and upregulation in specific sugars, amino acids and dehydrins (DHN), the exact nature of which varies among species [9•,10,30,31] (Figures 2bII *cf* 3bII). Photosynthesis continues, but in crops this metabolism is compromised on reaching a relative water content (RWC) of 60% when drought-induced senescence is initiated. ABA content increases during this stage in both models [24,32] and there is evidence of both ABA activated signalling processes

Figure 2

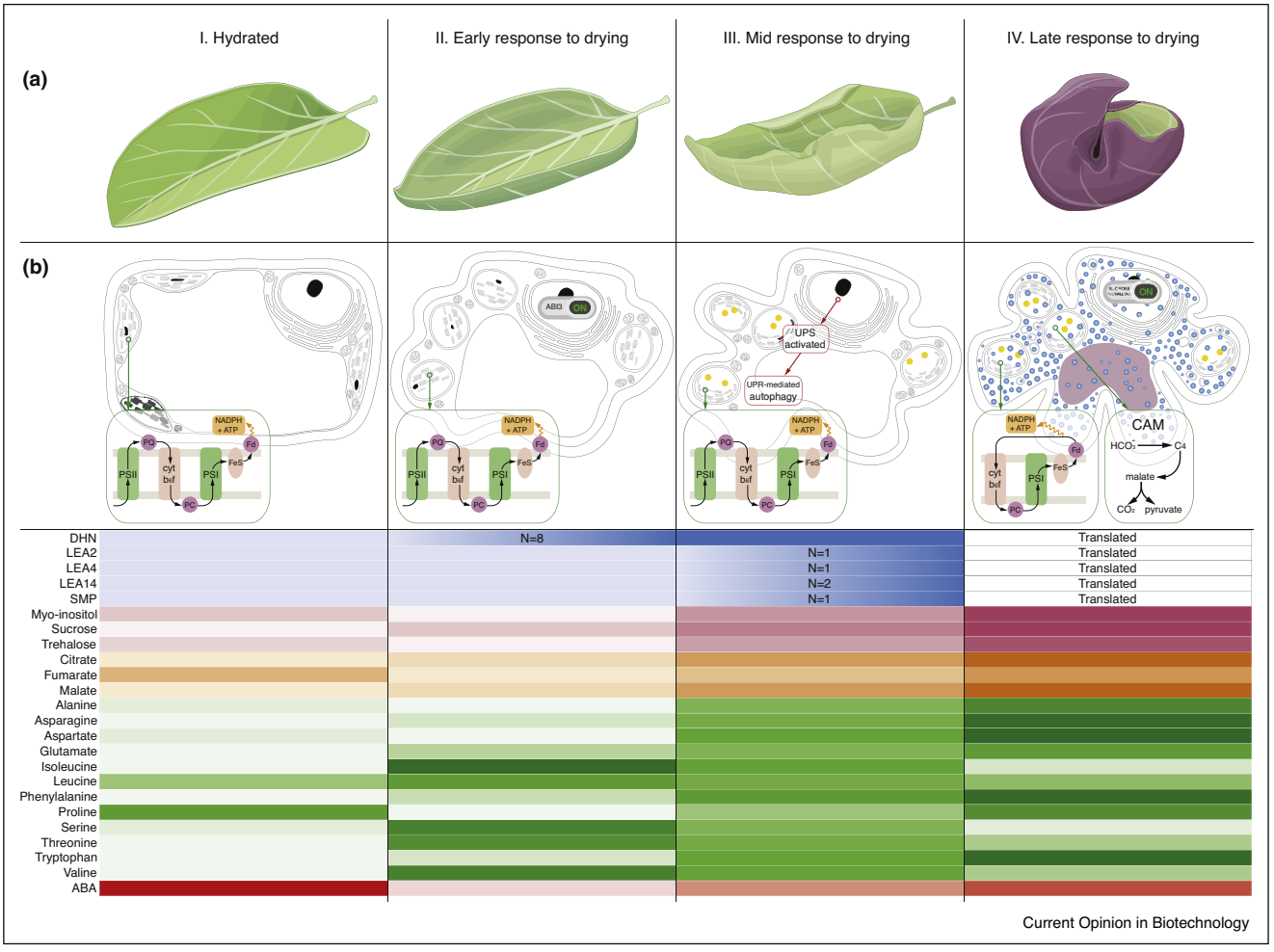


Schematic representation of changes in **(a)** leaf phenology and **(b)** subcellular organization (upper panel) and changes in key components (lower panel: LEAs, sugars, organic acids, amino acids and ABA) of a desiccation tolerant maize leaf during dehydration from full turgor (i) during the ERD, 100-60% RWC (ii), the MRD, 60-40 RWC (iii) and LRD, 40-5% RWC (i). Details were taken from Refs. [7,12,18–21,32,35]. LEA transcripts are shown in blue and the number of genes in each LEA family is noted within bars in heat maps in the lower panel. LEA proteins, present in the LRD only, are also shown in blue. ELIP transcripts, present in the LRD only, are shown in yellow in the lower panel. Sugars are shown in light pink, organic acids are shown in orange, amino acids are shown in green, ABA in red and anthocyanins in purple. The degrees of shading in the heat maps in the lower panel indicates changes in levels of each of the above compounds, with darker colours depicting significant increases and lighter colours significant decreases. The proposed ABI3 and ABI5 and sucrose switches are shown in the ERD and entry into the LRD respectively. The overall pink colour of the cytoplasm in the LRD is to depict considerable sucrose presence.

and a tempering thereof by activation of several members of the PP2C family of protein phosphatases [24,27••]. PP2Cs participate in regulation of seed dormancy in *Arabidopsis* [33] and we propose that genetic switches, such as the LAFL network referred to above, come into play in the ERD. Together with the priming nature of metabolite accumulation, this could serve as one of the master switches required for the induction of DT in crops (Figures 2b-II, 3b-II).

During the MRD (60–40% RWC) there are shifts in the nature of metabolism which differ among the models. In *X. schlechteri*, gas exchange ceases at 60% RWC and during this stage there is progressive degradation of chlorophyll and dismantling of thylakoids such that by the end of this stage chloroplasts resemble those present in dry seeds [21,23•] (Figure 2b-III). There is convincing evidence that even as such dismantling occurs, transcripts required for reconstitution of photosynthetic metabolism are transcribed and stably stored for translation on rehydration [15], similar to seeds. During this stage leaf blades fold, resulting in shading of the adaxial and exposure of the abaxial surfaces to light, these becoming anthocyanin rich, affording protection against potential ongoing

Figure 3



Schematic representation of changes in **(a)** leaf phenology and **(b)** subcellular organization (upper panel) and changes in key components (lower panel; LEAs, sugars, organic acids, amino acids and ABA (b) of a desiccation tolerant soybean leaf during dehydration from full turgor (i) during the ERD, 100-60% RWC (ii), the MRD, 60-40 RWC (iii) and LRD, 40-5% RWC (iv). Details were taken from Refs. [19,22,24,25,27••,29•,32,33,35]. LEA transcripts are shown in blue and the number of genes in each LEA family is noted in the bars in heat maps in the lower panel. LEA proteins, present in the LRD only, are also shown in blue in the upper panel. ELIP proteins, present in all stages of dehydration, are shown in yellow in the upper panel. Sugars are shown in light pink, organic acids are shown in orange, amino acids are shown in green, ABA in red and anthocyanins in purple in the lower panel. The degrees of shading in the heat map indicates significant changes in levels of each of the above compounds, with darker colours depicting significant increases and lighter colours significant decreases. The proposed ABI3 and ABI5 and sucrose switches are shown in the ERD and entry into the LRD respectively. The overall pink colour of the cytoplasm in the LRD is to depict considerable sucrose presence.

photosynthetic ROS production [23•] (Figure 2a-III). Anatomically, such folding is facilitated by presence of relatively thick cell walls which undergo some modification during the MRD [24,34] releasing sugars to the cytoplasm, which together with (increased presence of) amino and organic acids and anthocyanins, get partitioned into numerous small vacuoles enabling subcellular mechano-stabilisation [23•,24–26,27••,28,29•,30–35] (Figure 2b-III). There is increased transcription, but minimal translation, of 37 LEA genes from all LEA families and decreased transcription of many LEA_2s and DHNs [15,24] (Figure 2b-III). Respiration is ongoing and ROS damage prevented by sustained ongoing activities of ubiquitous antioxidant enzymes and increased levels of specific polyphenols [21,23•,35]. Importantly, during this stage drought induced senescence-associated processes are actively suppressed [24] although there is activation of the ubiquitin proteasomal system (UPS), proposed to be a less severe response than autophagy and allows for release of nutrients for protective purposes [9•].

In dicots, photosynthesis is maintained during the MRD with gas exchange and linear electron transfer ceasing only at 40% RWC [36] (Figure 3a-III,b-III). Photoprotection is afforded by non-photochemical quenching via the xanthophyll cycle carotenoids, particularly zeaxanthin, which together with α-tocopherol and early light-inducible proteins combine to protect the photosystems during this stage of dehydration [9•]. Considerable cellular shrinkage occurs during the MRD such that apical surfaces curl inwards (Figure 3a-III). Subcellular mechanical stabilization is achieved by increased wall folding (Figure 3b-III), this flexibility being enabled by pectin rich cell walls and expansin production [33,37]. The Calvin cycle continues to operate, but energy is differently partitioned so as to enable further accumulation of sugars and certain (different) amino acids [27••] (Figure 3b-III) which in turn 'buffer' the subcellular environment against the damaging effect of extreme water loss, preventing plasmolysis. At least 8* LEA genes are transcribed [25,27••] many seemingly different from those in *X. schlechteri* (Figures 3b-III, *cf* 2b-III). Respiration is ongoing with high levels of malate being accumulated, and as in the monocot model, the UPS is activated during this stage further contributing to the generation of metabolites required for desiccation tolerance [27••,30].

The LRD in both models is characterised by continued accumulation of stabilizing sugars (particularly sucrose) and amino acids, maintained antioxidant potential, translation of LEAs and HSPs, suppression of senescence and *de novo* transcription, and in some instances storage of transcripts required for recovery upon rehydration [15,23•,24,27••]. As before, they achieve the end result by employing players unique to each phenotype (Figures 2b-IV *cf* 3b-IV).

The most striking differences between the models is the maintenance of photosynthesis in dicots. During this stage dicots stop gas exchange, cease linear electron transfer, engage in cyclic electron transfer [28,36] and possibly CAM [27••]. Considerable leaf folding occurs exposing anthocyanin rich abaxial surfaces, these pigments being synthesised in the LRD (Figure 3a-IV, [35]). Another notable difference is the maintenance of respiration in Craterostigma to 20% RWC before a measured decline and a potential switch to the alternative respiratory pathway (AOX) occurs [27••,38]. In Xerophyta respiration declines from 40% RWC ceasing at 10%, but AOX is not engaged [21,24,38].

The most remarkable feature of the LRD is evidence of ongoing selective metabolisms at such extremely low water contents. It has been argued that this is made possible due to the existence of suborganellar and subcellular pockets of non-aqueous molecular mobility, enabled in part by the accumulation of metabolites that form natural deep eutectic solvents (NaDES) [39]. This hypothesis supports the notion of different types of metabolic activity occurring among species, enabled by and dependent on the biochemical nature of the subcellular milieu. This stage is terminated by tissues entering a biophysically vitrified state, brought about by massive accumulation of sucrose in combination with LEAs and HSPs [9•]. It is highly likely that sucrose signalling plays a role in the LRD [40] which, together with further ABA-mediated responses, enable metabolic quiescence, again synonymous with dormancy in seeds. We propose a second switch for ultimate commitment to DT at the start of the LRD (Figures 2b-IV, 3b-IV). In seeds longevity is acquired during the late stages of maturation, in terms of RWC comparable with the LRD[3], with ABA and the transcription factor ABI5 playing key regulatory roles [41]. This may explain why poikilochlorophyllous RPs like Xerophyta have greater longevity in the dry state than homoiochlorophyllous Craterostigma [39] as ABA contents at LRD are substantial in the former and low in the latter (Figures 2b-IV, 3b-IV).

The question arises whether poikilochlorophyllous resurrection species would also produce seed with greater longevity than homoiochlorophyllous species, and how this would reflect in ABA signalling in the seeds. Since the latter are mostly found in tropical areas there is no apparent need for long-term survival in dry conditions whereas the former must bridge longer dry periods, it seems plausible that the same goes for their seeds (in the soil). Would this imply that species with long-lived seeds have a greater potential to be transformed to become desiccation tolerant than short-lived? There is a huge variation in seed longevity across species and it is not clear yet how this is caused. Various factors seem to be involved, *inter alia* chemical composition, seed size and protection against oxidative damage.

Rehydration is rapid but recovery of full metabolic competence occurs within 48 hours in Craterostigma and 5–7 days in *Xerophyta* spp. [27••,42]. This is a consequence of their respective strategies to manage photosynthetic stress during dehydration. Interestingly, neither model requires *de novo* transcription or translation for initial recovery [27••,43,44,45]. A final but striking phenotype shown by resurrection plants is their ability to ensure flowering after drought, guaranteeing seed yield. Dicot flowers are desiccation tolerant and resurrect along with the plant. Monocots suppress flowering during drought, doing so immediately after rehydration (see Supplementary video).

[3] Determined from Refs. [22,24] and LEA classes depicted in Figure 3bIII were identified using National Center for Biotechnology Information (nih.gov).

Conclusion

The scenarios painted above, while seemingly complex and involving several levels of finely tuned regulation at the transcriptional, post-transcriptional, biochemical and even biophysical levels, may be as simple as 'flipping' seed-associated regulatory switches at the ERD and LRD; a feat that can be achieved by appropriate gene editing. This strategy, albeit extreme, guarantees survival of the harshest drought. Yield is unlikely to be perturbed in years with reasonable rainfall and some yield guaranteed in the face of an extreme drought. Thus of all 4 scenarios described above, creation of a resurrection phenotype will allow greatest expansion of agriculture into extreme environments (Figure 1).

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Both authors contributed equally towards conceptualization and writing of the article. **Henk Hilhorst** compiled Figure 1 and **Jill Farrant** conceptualized and wrote about Figures 2 and 3, which were compiled by **Keren Cooper** (see acknowledgments).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.copbio.2021.10.026.

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