protein of approx. 14 kDa, and this has been designated as a putative storage protein (JR14). Other stress-related proteins (dehydrins and smHSPs), which are often associated with tolerance of water deficits also increase. SDS-PAGE analysis of roots at all stages of the *R. asciaticus* life cycle revealed that JR14 accumulates during the desiccation period, and two-dimensional electrophoresis demonstrated that there are, in fact, two major proteins of around 14 kDa. One of these was purified, and the first 31 N-terminal amino acids were determined by sequencing. A 489 bp full length JR14 cDNA sequence was obtained by 3′-RACE and 5′-RACE PCR using oligonucleotide sequences based on the terminal amino acids. The amino acid sequence (163 amino acids) of the JR14 protein shows that it has stress-induced domains PLAT/LH2. A peptide of 24 N-terminal amino acids of the full length JR14 protein is a possible signal peptide, indicative that it is a sequestered protein. It has no strong similarity with known water-stress-related proteins, nor with the low mol. mass storage protein present in the tap roots of dandelion. Western blots indicated that the expression of dehydrin Dhn4B (LEAII D-11 family) is induced by dehydration and ABA, and declines during rehydration. The cDNA sequence of *Dhn4B* has a very high similarity with dehydrins known in other species. As with dehydrins, smHSPs were also detected by western blots in the tuberous roots during desiccation, and their expression was induced by high temperature and dehydration.

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The effect of developmental status on successful cryopreservation of recalcitrant seed germplasm

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The zygotic germplasm of plant species producing desiccation-sensitive seeds can be conserved in the long-term only by cryopreservation. Usually the embryonic axis is excised from the cotyledons and is used as the explant for cryopreservation, as it provides a favourable surface area:volume ratio. However the shoot of the axis of most species studied does not develop after excision, with the result that survival after cryopreservation is often recorded as callus production or simply greening. Thus, besides explant size, factors such as *in vitro* regeneration techniques, physical damage induced upon excision and developmental status of the seed could compromise the success of cryopreservation. This study investigated the effect of the factors mentioned above, with particular attention to the developmental status of the seeds, on *in vitro* development and cryopreservation of the desiccation-sensitive embryonic axes of three species: *Trichilia dregeana*, *T. emetica* and *Strychnos gerrardii*. For all three species, investigations were conducted on the embryonic axes excised from mature seeds immediately after harvesting and from mature seeds stored under hydrated conditions for different periods (in order to achieve different degrees of development). When germinated *in vitro*, excised embryonic axes of *T. dregeana* and *T. emetica* did not develop shoots unless the axes were excised with attached cotyledonary segments. Following the development associated with short-term storage, however, the axes could develop normally without any attached cotyledon. The embryos from the (endospermous) seeds of *S. gerrardii* developed normally without any attached cotyledon, but the percentage germination increased with seed storage period. For all three species, *in vitro* axis germination was optimal when activated charcoal was included in the germination medium, regardless of the developmental stage of the seeds. On dehydration to approximately 0.3 g g^{-1} (dry mass), embryonic axes from all three species failed to develop shoots even though more than 50% of explants produced roots. Hence, shoot production was more sensitive to desiccation than was root production. The sensitivity of shoots to desiccation did not change with seed storage for *T. dregeana* and *T. emetica*, but decreased for *S. gerrardii* when the seeds were stored for 6–8 weeks. Subsequent cryopreservation of *T. dregeana* and *S. gerrardii* explants was best achieved with rapid cooling in nitrogen slush (−210°). However, the optimal cooling procedure for successful cryopreservation of *T. emetica* explants is still to be established. The highest post-cryopreservation survival of *T. dregeana* axes was achieved when seeds had been stored for three months, while the seed storage period did not affect post-thaw survival of the axes of *T. emetica* and *S. gerrardii*. Only *S. gerrardii* explants produced shoots after cryopreservation, whereas the surviving embryonic axes of *T. dregeana* and *T. emetica* regenerated only as non-embryogenic callus. Thus, the successful cryopreservation of the germplasm of the species tested, and others producing recalcitrant seeds, depends on numerous species-specific factors.

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Seedling survival after desiccation when rehydrated with NaCl solutions, in some annual and perennial desert halophytes

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Seedling of the perennial desert halophytes *Haloxylon aphyllum*, *Salsola dendroides*, *Kochia scoparia* and *Kochia prostrata*, of the Chenopodiaceae family, from the Solonchak salinities of Kyzylkum desert in Uzbekistan were tested for their survival when germinated and rehydrated with NaCl solutions after periods of desiccation. The seeds were tested for germination in distilled water as well as in NaCl solutions of up to 3%, in light and darkness. The seeds germinated well in

NaCl solutions of up to 2% and some up to 2.5%, but none germinated in 3% NaCl. After wetting for 24 h, the germinating seedlings were transferred to dry filter paper and desiccated under room conditions (20 to 25 °C and 15 to 20% R.H.) for 14 or 30 days. Then the seedlings were rewetted, each treatment with its original solution; seedlings that continued to grow after rewetting were counted as seedlings that had survived desiccation. Three of the four species were found to have seedlings with nearly 100% of desiccation tolerance. Even after 30 days of desiccation, seedlings continued to grow when rewetted with solutions of up to 2% NaCl. Only *K. prostrata* had low percentages of survival after desiccation. *Schismus arabicus* is one of the most common annual plants species inhabiting large areas of the extreme dry and saline desert in the Saharo–Arabian and Irano–Turanian regions of the Negev and Judean deserts of Israel. This species is one of the first plants in the Negev to germinate, even after small amounts of rain of about 10 to 15 mm. *S. arabicus* seeds (caryopses) may start to germinate within 6 h of wetting under optimal laboratory conditions. The ability of *S. arabicus* seeds to germinate and seedlings to survive long periods of desiccation was established when germinated and rewetted with distilled water. In the current sets of experiments it was found that *S. arabicus* seeds may germinate well in up to 2% NaCl and some germinate in 2.5% NaCl. Such seedlings may renew their growth and elongation after a period of desiccation when they are rewetted with solutions of up to a concentration of 2% NaCl. Such abilities of seedlings are not common but represent an important survival strategy for species inhabiting extreme dry and saline deserts with unpredictable amounts and distribution of rain.

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The use of the model species *Medicago truncatula* in studies on desiccation tolerance and sensitivity in seeds

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While many studies have been devoted to reveal the secrets of desiccation tolerance (DT) in seeds, less effort has been put into clarifying the recalcitrance phenomenon. The research on recalcitrant seeds faces a number of obstacles, the main one being the very limited time of availability of fresh seeds. Tree species can make the situation even more difficult, since they can alternate years of massive seed production with years of low or no production. The acquisition of more fundamental knowledge of the recalcitrance phenomenon is crucial for the advancement of *ex situ* conservation of germplasm from endangered species. Only with successful *ex situ* conservation protocols will the biodiversity of recalcitrant species be preserved. The non-existence of a model system for studies on recalcitrance can account for the slow progress on this subject. Thus, it has been suggested that germinated orthodox seeds can be used as a model system for studies on recalcitrance, based on the fact that upon germination, orthodox seeds lose DT progressively, becoming comparable to the recalcitrant types. Many processes, at the physiological, cellular and molecular levels, involved with the loss of DT in germinated orthodox seeds, may be similar to those responsible for the desiccation sensitivity (DS) shown by recalcitrant seeds. Another advantage of working with germinated orthodox seeds is the feasibility of the re-establishment of its DT through the use of relatively simple techniques (such as osmotic stress and exogenous ABA). Such a system has thus the great advantage of allowing comparisons among different degrees of DT and DS within the same species, and appears as an outstanding tool for studies on the mechanisms responsible for these traits in seeds. The model species *Medicago truncatula* Gaertn. (Leguminosae, Papilionoideae), originally chosen for studies on symbiotic nitrogen fixation, produces orthodox seeds and for this reason can be useful for studies on seed biology. We have used this species in studies on DT/DS and the results have shown that it is a promising model system for such studies. By incubating germinated seeds in polyethylene glycol (PEG) it was possible to re-establish DT in radicles up to 2 mm long. Flow cytometric analyses showed that up to this length, there is no DNA synthesis, which was first detected in radicles with a length of 3 mm. Immunohistochemical detection of microtubules (MTs) showed that dehydration of germinated seeds with a 2 mm radicle, with or without PEG treatment caused disassembly of MTs and appearance of tubulin granules. Upon rehydration, neither MTs nor tubulin granules were detected in radicle cells of untreated seeds, while PEG-treated seeds were able to reconstitute the microtubular cytoskeleton and continue their normal development. Dehydration of untreated germinated seeds also led to an apoptotic-like DNA fragmentation in radicle cells, while in PEG-treated seeds DNA integrity was maintained. Studies on gene expression in radicles showed that transcript abundance of DT-related genes decreased during germination and increased by osmotic treatment, correlating with the loss and reacquisition of DT. All together, our results suggest that the occurrence of the first cell cycle appears to be an overriding factor that abolishes re-establishment of DT.

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Desiccation sensitivity of *Antiaris toxicaria* axes and reactive oxygen species scavenging enzymes in mitochondria

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Axes of *Antiaris toxicaria* seeds and washed mitochondria isolated from the axes were used as experimental materials in