Desiccation tolerance of yeheb (*Cordeauxia edulis* Hemsl.) seeds

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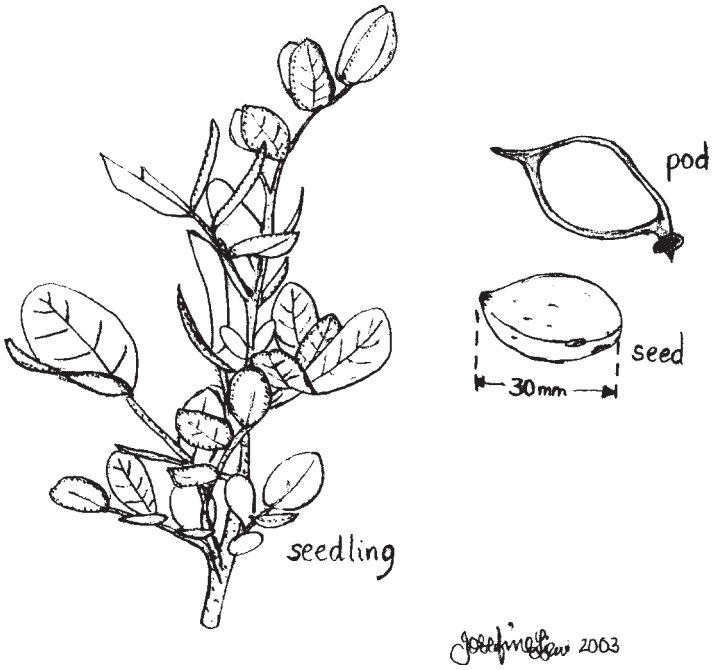

Examensarbeten/Seminarieuppsatser • 63

Institutionen för ekologi och växtproduktionslära

Uppsala 2003

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Uppsala 2003

ISSN 1404-0409

Examinator: Margareta Hansson
Handledare: Lars Andersson

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Abstract

Liew, J. (2003) Desiccation tolerance of yeheb (*Cordeauxia edulis* Hemsl.) seeds. SLU, Department of Ecology and Crop Production Science. Master thesis, 63. Uppsala, Sweden.

In this master's thesis, the desiccation tolerance of seeds from yeheb, *Cordeauxia edulis* Hemsley, a multipurpose, evergreen shrub, native to the semi-desert areas in the horn of Africa, is studied. Due to overexploitation and bad regeneration, yeheb is now threatened with extinction.

Seeds of different species can be classified into three storage behaviour categories, depending on their desiccation tolerance, longevity and tolerance to low temperatures during storage. When determining the storage behaviour of seeds, the first step is to study desiccation tolerance. Seeds that maintain their viability at <5% moisture content (wwb=wet weight basis), are probably orthodox. If they tolerate 10-12.5% moisture content (MC), but not lower, they may be intermediate, and if most seeds die when desiccated to moisture contents >15-20%, then they are recalcitrant. Desiccation tolerance of seeds is controlled by several physiological processes, and depends on factors such as drying rate, tissue, degree of development at harvest and environmental influence of the mother plant prior to seed maturation/harvest.

To achieve the desiccation of seeds, silica gel was used. The seeds were dried to six different moisture contents: 9.6, 12.3, 24.4, 27.4, 33.4 and 39.3% (wwb). Moisture content of the fresh seeds was 41.6%. Desiccation tolerance was assessed by germination tests followed by a tetrazolium test of viability on ungerminated seeds that were not estimated to be clearly dead at the end of the germination test. Germination tests were done on desiccated and fresh seeds, and on seeds from 'control of time factor during drying' -replications, in which vermiculite were used instead of silica gel during the drying treatment. Germination percentage was dependent on seed moisture content (p-value 0.0001), seen as a reduction of germinability when seed moisture content dropped from >24.4 to 12.3% (p-value 0.0062). Further drying to 9.6% moisture content, reduced germination percentage even more (70-83.8% at MC >24.4%, 57.5% at 12.3% MC and 41.3% at 9.6% MC). The reduction of germination capacity at the latter MC was highly significant compared to the control replications (p-value 0.0001). Yeheb seeds may therefore be classified as having seeds of intermediate storage behaviour. However, further studies on viability in storage and tolerance to low temperatures are necessary before any certain conclusions on the classification of seed storage behaviour of yeheb can be drawn.

Sammanfattning

Liew, J. (2003) Torktolerans hos frön från yeheb (*Cordeauxia edulis* Hemsl.). SLU, Institutionen för ekologi och växtproduktionslära. Examensarbete, 63. Uppsala.

I det här examensarbetet studeras torktoleransen hos frön från yeheb, *Cordeauxia edulis* Hemsley, en användbar, städsegrön buske, endemisk för semi-arida ökenområden på Afrikas Horn. På grund av överexploatering och bristande reproduktion hos bestånden, är yeheb utrotningshotad.

Frön från olika växtarter kan delas in i tre olika lagringsbeteendekategorier, beroende på tolerans mot torkning, låga temperaturer och livslängd vid lagring. Det första steget vid klassificering av frönas lagringsbeteende, är att undersöka deras torktolerans. Frön som tål <5% vattenhalt (våtviktsbasis) med bibehållen livsduglighet är förmodligen ortodoxa. Om de tål nedtorkning till 10-12.5% vattenhalt (vh), men inte lägre, är de troligen intermediära och om de flesta frön dör vid vattenhalter över 15-20%, är de recalcitranta. Torktolerans hos frön kontrolleras av flera fysiologiska processer, och är beroende av faktorer som torkningshastighet, vävnad, utvecklingsgrad vid skörd samt moderplantans miljö innan frömognad/skörd.

För att torka fröna till sex olika vattenhalter: 9.6, 12.3, 24.4, 27.4, 33.4 och 39.3% användes kiselkorn. Vattenhalten hos färska frön var 41.6%. Torktoleransen utvärderades genom groningstest följt av ett tetrazoliumtest av livsduglighet på ogrodda frön som inte klart kunde bedömas vara döda efter groningstesten. Groningstest gjordes på färska och torkbehandlade frön samt frön från 'en test av tidsfaktorn', där vermikulit ersatte kiselkorn under torkningsbehandlingen. Groningen var beroende av frönas vattenhalt (p-värde 0.0001), vilket kunde ses som minskad groning när frövattenhalten minskade från >24.3 till 12.6% (p-värde 0.0062 jämfört med kontrollerna). Groningen försämrades ytterligare när vattenhalten minskade till 9.6% (70-83.8% grodda frön vid vattenhalt >24.4%, 57.5% vid 12.3% vattenhalt och 41.3% vid 9.6%). Jämfört med kontrollerna var groningsreduktionen vid 9.6% vattenhalt starkt signifikant (p-värde 0.0001). Yeheb skulle därför kunna klassificeras som en art med intermediära frön. Dock är det nödvändigt med studier av livslängden vid lagring samt tolerans mot låga temperaturer innan det är möjligt att dra säkra slutsatser om yehebfrönas kategoritillhörighet med avseende på lagringsbeteende.

Ämnesord: Agrovoc: *Cordeauxia edulis*, nut crops, seed moisture content, desiccation, viability, Somalia, Ethiopia.
Egna: recalcitrant, orthodox

Desiccation tolerance of yeheb (*Cordeauxia edulis* Hemsl.) seeds

Yeheb seedling, about 2 months after sowing.

Desiccation tolerance of yeheb (*Cordeauxia edulis* Hemsl.) seeds

Introduction

Yeheb, *Cordeauxia edulis* Hemsley, is an evergreen shrub (Ali, 1988), native to the semi-desert areas in the Horn of Africa (Vietmeyer, 1985). It was identified as a Fabaceae (Leguminosae) belonging to the tribe Caesalpiniaceae (Caesalpiniodeae), and is the only species in the genus Cordeauxia (Bally, 1966). *C. edulis* is a wild plant with multipurpose usage. The seeds and the leaves are important source of food and fodder during the dry periods (Bally, 1966; Kazmi, 1979; El-Zeany and Gutale, 1982; Ali, 1988; Zimsky, 1990) and the hard wood provides fuel for cooking (Vietmeyer, 1985). In addition, the red pigment, cordeauxiaquinone, in glands on the leaves are used as a textile dye (Bally, 1966). The leaves are also infused to make a tea (Greenway *et al*. 1947).

Over-exploitation by over-grazing and seed harvesting due to wars and periodic droughts during the last decades, have resulted in a decline in yeheb populations over most of its former habitat, where it was once the dominant shrub (Vietmeyer, 1985; Ali, 1988). Therefore, yeheb is threatened with extinction (Bally, 1966; Kazmi, 1979; Nerd, 1994), and is listed as endangered by International Union for Conservation of Nature (IUCN, 1997). Efforts have and are being made to domesticate the species and also to introduce it outside its natural habitat (Bally, 1966; Kazmi, 1979; Nerd, 1994). There are, however, some obstacles when cultivating yeheb, such as a fast tap root growth, poor seed supply, lack of agronomic techniques and water necessary for establishment (Yahya, *pers. comm.*).

Because of the above mentioned obstacles, a project on domestication of yeheb have been initiated by the Swedish University of Agricultural Sciences, Department for Ecology and Crop Production Science, in co-operation with Alemaya University in Ethiopia. The aim is to find ways to propagate yeheb by seeds and seedlings and establish in situ conservation of gene resources within its natural habitat and at proper ecological sites in eastern Ethiopia. To achieve the propagation by seeds, it is important to store the seeds from one season to the next. The objective of this study is to investigate the desiccation tolerance of yeheb seeds, an important issue for the storage conditions chosen when storing seeds for long-term preservation and regeneration.

Literature review

Yeheb (*Cordeauxia edulis* Hemlsey)

Botanical and morphological description

Yeheb, *Cordeauxia edulis* Hemsley, is an evergreen, multi-stemmed and thick crowded shrub (Ali, 1988), usually dwarf and not taller than 1,6 m because of grazing of tender shoots and leaves by livestock. In sheltered locations, where the plants are not grazed, yeheb can reach 3-4 m and is then classified as a small to medium sized tree (Kazmi, 1979). Plants that are heavily browsed resprout profusely from the base. Yeheb is single stemmed in the early stages of growth. The leaves are pinnate, 2-8 cm long, with 2-4 pairs of leaflets that are oval to oblong, 1.7-3.6 cm long and 0.43-2.0 cm wide (Ali, 1988). They are leathery, olive green above and lighter on the underside with numerous purple to orange red glandular hairs on both sides (Bally, 1966; Miège and Miège, 1978; Ali, 1988). The glands contain a red pigment, cordeauxiaquinon, which by handling or when touched stains the hands red (Lister *et al*, 1955; Bally, 1966; Yahya, *pers. comm*.). Stomates occur both on the adaxial and abaxial surfaces of the leaflets. Both sides of the leaf are covered by an extremely thick cuticle. All of the mesophyll cells consist of 'concertina' palisade cells. These are suggested to shrink under water stress, and thereby causing the cell walls to fold (Curtis *et al*, 1996), which leads to curling of the leaves. This happens as soil moisture content drops. At the onset of the first rain, indicating the end of the drought period, the leaves uncurl and regain their turgidity (Ali, 1988). The leaves exist for more than one season, but the lifespan of individual leaflets are not known (Curtis *et al*, 1996).

Floral and vegetative auxillary buds develop apically and laterally from the branchlets (Ali, 1988). The inflorescence is a few-flowered corymb that forms at the end of the branchlets. The flowers are 2-5 cm in diameter, with five bright yellow, spatular petals, 1.5 cm long and five green sepals, which are about 1 cm long and covered with red glands, like the leaves. The ten stamens are hairy at the base of the filaments and the ovary is shortly stalked, heavily glandular and surmounted by an obtuse stigma (Bally, 1966; Miège and Miège, 1978). Flowering occurs throughout the year, but is more profusely during the rainy season. The fruit, which is a pod, is 4-6 cm long, rather leathery and compressed ovoid. The walls are fairly hard and terminate in a protuberant break. Each pod contains 1-4 seeds (Miège and Miège, 1978). The seed weights about 2-3 g each (TSW 2000-3000 g) (Nerd *et al*., 1994).

The root system is dominated by a central taproot, which reaches a depth of >2 m. Lateral roots form 10 and 40 cm below the soil surface, and spread horizontally a distance of 2.3 m according to Ali (1988).

There are two forms of C. edulis, a small-leaf form, locally called Suuley, and a large-leaf form, called Muqleey. Generally, these do not grow in mixed stands. Suuley produces pods with a single, large sized seed, which according to the nomads, have a sweeter taste

than seeds from the Muqleey-form, whose pods contain more than one, but smaller seeds (Ali, 1988). A chemical analysis of the two forms shows a somewhat higher sucrose content in Suuley compared with Muqleey (20.1% and 19.5% respectively). Muqleey produces rounded, flat sided seeds, Suuley ovoid seeds (El-Zeany and Gutale, 1982). It takes 3-4 years from germination until the plant begins to bear seeds (Kazmi, 1979). The number of chromosomes are 2n=24 (Miège and Miège, 1978).

Seedling development

Miège and Miège (1978) report that yeheb seedlings have poor growth of aerial parts during the early stages of development, but the root system grows very rapid. The tap root pushes deeply into the ground, exploiting as much of the water reserves as possible. The germination is epigeous. The seedling sprouts with thick cotyledons, number of leaflets varying between individual plants. The leaves are alternate. Yahya (*pers. comm*.) found that a 15 days old seedling had a 2 cm tall shoot and a tap root of 32 cm.

Site conditions

The native region of yeheb is the semi-desert region in the Horn of Africa, where rainfall is only 150-200 mm a year (Vietmeyer, 1985). The rainfall in the natural habitat of yeheb is bimodal, in which there are two main rainy seasons, of varying reliability in April-June and October-November, and two pronounced dry seasons (A. Yahya, *pers. comm*.). Characteristic yeheb-soils are deep and salt-free soils of red Haud sands in the northeastern corner of Ethiopia and the "Haud-type" soils in Somalia, northeast of Mataban, around this town and southeastwards to Jesomma. The soil has low levels of exchangeable cations, and also low levels of Zn, B, P and N, which indicate poor fertility. pH ($CaCl_2$) is 7.27-7.34, uniformly high throughout the soil profile. The Fe-levels are high, and lime is absent (Drechsel and Zech, 1988).

Bally (1966) reports that in 1954, yeheb was found in areas consisting of open bush savannas, where yeheb was dominant. Apart from a few scattered trees, yeheb was the tallest of the woody plants. Yeheb-plants grew in scattered, but dense clumps with much open tufted grasslands between them. Most of the mature plants bore traces of being browsed upon by goats. Ali (1988) found that yeheb-stands were poorer near to permanent water points and villages where livestock and people densities were high. According to Kazmi (1979), yeheb grows in open bush savannas at altitudes of 300-1000 m. These areas are forest free and have two rainy seasons a year and an annual rainfall of 250-400 mm. Zimsky (1990) reports that yeheb requires a yearly rainfall of 250-400 mm, and can survive on as little as 150 mm at an altitude of 300-1000 m.

Biology

Yeheb starts new vegetative and floral development, as well as continue vegetative and reproductive development initiated the former rainy period, at the onset of the rainy season. The flowers develop to the first stages of fruit formation, but then go into a diapause stage, in which they remain throughout the dry season. In the next rainy period, the fruits continue their development and mature within a few days. Unlike most plants, seeds of yeheb mature when the plant is at its highest water content. According to the nomads, seeds do not require water to germinate immediately after harvest and will germinate in storage if not immediately dried. The leaves contain less than 30% water in the dry season, and the leaf water potential may be such that moisture can be extracted from the atmosphere in the humid period prior to the rainy season (Ali, 1988).

The natural way of propagating yeheb seeds is brought about with help of squirrels, which first eat seeds until they have satisfied their hunger, then bury the remaining fruits in the soil. Yeheb is threatened with extinction because of competition between humans and squirrels for the seeds as food and the many other uses that other parts of the shrub has. When people have harvested fruits for their needs and squirrels have had enough to eat, no seeds remain to be buried, and therefore, no propagation is achieved (Yahya, *pers. comm.*).

Johnson (1996) reports the yeheb-seeds to be recalcitrant, losing viability after four to six months, which is an unusual trait among arid-land legumes. However, no evidence for this conclusion was present in the article.

Economic value and usage

Seeds from yeheb, which are sometimes called nuts, are eaten fresh or boiled, occasionally roasted. They are mostly consumed locally and are considered as a staple food for poorer people. The seeds are also sold on the local market and exported to the coastal area, where they are much in demand among people. The leaves, containing the red pigment cordeauxiaquinone, are used as a textile dye (Bally, 1966). The leaves can also be infused to make a tea (Greenway *et al*. 1947). The water in which the seeds have been boiled, is sweet and is sometimes used as a beverage (El-Zeany and Gutale, 1982). According to Kazmi (1979), the yeheb-seeds are the staple food of the nomads living in, or migrating trough the Somali hinterland. Ali (1988) reports that the nomads compete with each other for the yeheb seeds, which have led to pre-harvesting, before the seeds are physiologically mature, fearing someone else may harvest ahead of them.

The leaves and tender shoots are grazed upon, providing feed for livestock, as camels and goats, especially during the end of the dry period (Assefa *et al*., 1997). Wildlife, especially baboons also eat the seeds (Ali, 1988). The hard wood is used as fire-material for cooking (Vietmeyer, 1985). The twigs of the aerial plant are also used as constructing material for houses. As yeheb is resistant against termite attacks, houses and sheds made

of yeheb persist for decades. The plant also protects the soil from erosion (Yahya, *pers. comm.*).

Nutritional value

The fodder quality of yeheb leaves is comparable to other tropical tree legumes (Drechsel and Zech, 1988) and during the dry period, the forage quality is considered as high relative to other plants (Ali, 1988). Some minerals, however, would not satisfy the demand of animals if yeheb were the only source of fodder. Foliar N is low, and crude protein is lower than would be expected for a probably N-fixing Sahelian plant. This has lead to the suggestion that yeheb has limited capability for nitrogen fixation (Drechsel and Zech, 1988). Research by Assefa *et al*. (1997) supports this theory, as they did not found any nodulation of either on-site excavation of plants at the beginning of the short rainy season in April, or of plants inoculated with rhizobia strains isolated from Ethiopean woody legumes.

The seeds do contain non-nutritious trypsin inhibitors, but the more toxic lectins, like phytohemagglutines, are absent. Compared to other legumes, the seeds are methionine deficient (Miège and Miège, 1978). The yeheb seed protein is rich in essential lysine and arginine, but deficient in tryptophan and isoleucine. Seeds with 17% moisture content consist of ca 13% protein, 10-11% lipids, 31-34% starch, 20% sucrose, 2% reducing sugars, 3% ashes and 2% fibre. The major components of the fatty acids are palmitic, oleic and linoleic acid together with stearic acid (26-31%, 32%, 25-30% and 12-13%, respectively). There are only traces of linolenic acid. The oil is a non drying oil (El-Zeany and Gutale, 1982). The same authors state that yeheb seeds make an unusual balanced diet, especially in areas where the usual legumes are impossible to grow.

Names

C. edulis is a multipurpose plant with several names. Bally (1966) reports the following spellings: ghieheb, giaeb, giaheb, gieheb, ieebb, iee-ep, ieheb, iieb, jeebb, jieheb, ye'eb, yee-ep, yeheb, yehib. Phonetically they express very much the same sound, although.

Why study desiccation tolerance of seeds?

The necessity of seed storage was firstly recognised when humans began to domesticate plants thousands of years ago. Viable seeds had, and still have, to be maintained from one growing season to the next. The goal of gene banks is to maintain seed viability for indefinite periods of long term storage, typically for 10 to 100 years or more. Seed longevity varies among, and within species, because of differences in genotype and provenance. The cumulative effect of the environment during seed maturation, harvest, drying and pre-storage, the time of seed harvest, duration of drying and the period that has passed before seeds are placed in storage influence the potential longevity of the

seeds. Based on their dehydration tolerance and storage longevity, in other words, their storage behaviour, seeds are classified into three groups: orthodox, intermediate and recalcitrant. When determining the most suitable storage environment and estimating the duration of successful storage, it is essential to know to which of these categories seeds from a particular species belongs (Hong and Ellis, 1996).

As dehydration tolerance is one of the factors on which the classification depends, seed desiccation tolerance, or desiccation sensitivity for different species is important to investigate.

Seed storage behaviour

As mentioned above, seeds are classified into three groups, orthodox, recalcitrant and intermediate based on the tolerance of desiccation and behaviour in storage. There is a wide range in the post-harvest responses of seeds, which suggest an open-endedness to the three categories. The post-harvest physiology of seeds may therefore be considered as constituting a continuum across species (Pammenter and Berjak, 1999).

Orthodox seeds

Roberts (1973) defined orthodox seeds as seeds in which the period of viability may be extended in a predictable way by lowering the temperature and moisture content during storage. The moisture content may be reduced to 2-5% or even lower before drying ceases to increase the viability period (Table 1). Ellis *et al*. (1989) suggest this critical moisture content to correspond to moisture content in equilibrium with 10% RH (relative humidity) for twelve orthodox species, which corresponds to a water potential around -350 MPa. Species whose seeds are orthodox can be maintained satisfactorily *ex situ* over long time if the environment is appropriate. All orthodox seeds, independent of provenance, store best at subzero temperatures (Hong and Ellis, 1996). Examples of species with orthodox seed storage behaviour are *Hordeum vulgare* L.*, Triticum aestivum* L. (Roberts, 1973), *Sterculia setigera* Del. and *Brassica napus* L. (Leprince *et al.*, 1998). According to Roberts (1973), most species have seeds belonging to this storage category.

Table 1. Properties of seeds depending on storage category. Critical moisture contents of seeds are based on fw (fresh weight)

	critical moisture contents fw of seed	water potential	Storage temperature	longevity in storage
Orthodox	2-5%	-350 MPa	<0°C	several years
Recalcitrant	23-61.5%	-1.5--5 MPa	cold or 10-15°C	2 weeks-3 years
Intermediate	5-12%	-90--250 MPa	cold or 10-15°C	½-6 years

Desiccation is considered necessary for the completion of the life cycle in orthodox seeds. This so called maturation drying is the third and final stage in the seed development, which starts by fertilisation, cell division and histodifferentiation of the major tissues during the first phase. The second phase involves accumulation of storage

compounds, such as lipids, starch and proteins. This stage is characterised by an increase in seed dry weight. Before the seeds begin to loose water, they acquire mechanisms that provide them with the ability to withstand dehydration. Therefore, orthodox seeds tolerate low moisture contents (Leprince *et al*., 1993).

Water sorption isotherms describe the relationship between water content and water potential (Walters *et al*., 2001). For orthodox seeds and desiccation tolerant tissues, the sorption isotherms follow a typical reverse sigmoidal shape (Vertucci and Leopold, 1987a, b, Eira *et al*., 1999).

Recalcitrant seeds

Roberts (1973) also described species with recalcitrant seed storage behaviour. In these seeds a decrease in moisture content below some relatively high value between 12-31% moisture content, depending on species, tends to decrease the period of viability. According to Hong and Ellis (1996), the critical moisture content for viability not to be lost in recalcitrant seeds varies between 23 to 61.5% on a wet weight basis. According to Sun and Liang (2001), the critical moisture content, expressed in terms of critical water potentials, for recalcitrant seed tissues from different species varies between -4.3 MPa (axis from *Bruguiera cylindrica*) and -10.8 MPa (axis of *Theobroma cacao*). The dehydration was achieved by using the equilibrium dehydration method, in which the seed tissues were equilibrated with solutions of known RH or water potential values, and then desiccation damage was measured. According to Hong and Ellis (1996), the critical water contents for recalcitrant seeds are moisture contents in equilibrium with 96-98% RH, corresponding to a seed water potential of -1.5 to -5 MPa.

Recalcitrant seeds do not undergo any maturation drying during the final phase of development. Their development continues after shedding, which in ultrasturctural investigation of meristematic cells of *Camelia sinensis* was indicated by metabolic activity in the most quiescent state immediately after harvest (Berjak *et al*., 1989). However, Probert and Hay (2000) suggest that *C. sinensis* seeds are intermediate, but no references to this proposal were apparent in the article. Recalcitrant seeds are often chilling sensitive (Berjak *et al*., 1989). The same authors suggest that all seeds that fail to meet the criteria for orthodoxy, that is, are neither shed in the dry state nor can be dried and successfully maintained in this state at moderate temperature for periods of months to years, will be considered recalcitrant. However, a third category of storage behaviour, namely the intermediate seed storage behaviour, has been described later (Ellis *et al*., 1990, see below).

There are varying degrees of recalcitrance. As reviewed by Berjak *et al*. (1989) there is a continuum of recalcitrance among species. The species may be grouped as showing a minimum, moderate or a high degree of recalcitrance. Seeds in the first category tolerate a fair amount of water loss and relatively low temperatures. Ecologically, these species will be found in temperate and sub-tropical regions. The highly recalcitrant seeds are produced by tropical forest and wetland species. Those seeds tolerate only a little water

loss, and are generally temperature-sensitive. Species with moderate recalcitrance tolerate desiccation and temperatures intermediate to the two other groups, and are distributed in the tropics.

For practical storage, species with recalcitrant seeds can be subdivided into two groups: those of tropical origin, which do not tolerate temperatures below 10-15°C, and those adapted to temperate climates, which can be stored at cooler temperatures for longer periods. All recalcitrant seeds must be stored in a more or less moist state. Another way of storage is cryopreservation of somatic and zygotic embryos (Hong and Ellis, 1996). Storage lifespan varies among species, from 2-3 weeks for tropical and 2-3 years for low-temperature storage of chilling tolerant species (Pammenter and Berjak, 1999). Examples of species with recalcitrant seed storage behaviour are *Avicenna marina* Forssk. (Berjak *et al*., 1989), *Quercus robur* L. (Finch-Savage, 1992), *Shorea robusta* Gaern. f. (Chaitanya and Naithani, 1998), *Boscia senegalensis* (Pers.) Lam. ex Poir., *Butyrospermum parkii* (G. Don) Kotschy, *Cordyla pinnata* Lepr. (ex Rich) M.-Redh, *Saba senegalensis* (A. DC.) Pichon (Danthu *et al*., 2000), *Aesculus hippocastanum* L. and *Theobroma cacao* (Probert and Hay**,** 2000).

Water sorption isotherms for recalcitrant seeds and desiccation-intolerant tissues are hyperbolic (Vertucci and Leopold, 1987b).

Intermediate seeds

It was Ellis, Hong and Roberts (1990) that first proposed a third category of seed storage behaviour. They found that seeds from cultivars of arabica coffee, *Coffea arabica* L. withstood desiccation to between 5-10%, corresponding to a water potential of approximately -90 MPa to -250 MPa. In all of the four investigated cultivars, seed longevity at cool and sub-zero temperatures in combination with low moisture content resulted in a more rapid loss of seed viability than at warmer temperature or higher moisture content. This was inconsistent with an orthodox, and a recalcitrant seed storage behaviour, which lead to the suggestion that a third, intermediate storage behaviour could be distinguished. One of the characteristics of this category would be that the dry seeds are injured at low temperatures. They also suggested that the optimum storage condition for these kinds of seeds would be at temperatures around 15°C with a moisture content slightly greater than 10%.

According to IPGRIs protocol to determine seed storage behaviour (Hong and Ellis, 1996), the intermediate seed storage behaviour is characterised by the reverse of the negative relation between seed longevity in air-dry storage and moisture content. This occurs at values below those of seeds in equilibrium with about 40-50% RH. Often, intermediate seed behaviour is associated with damage immediately after desiccation to relatively low moisture contents, about 7-12% (Hong and Ellis, 1996). Seeds from *Azadirachta indica* A. Juss. tolerated moisture contents of 4% before seed viability was reduced (Leprince *et al*., 1998). The longevity of the dry seeds is reduced if temperature drops below 10°C for species with intermediate seeds and of tropical origin. When

deciding the appropriate storage conditions for intermediate seeds, it may be helpful to distinguish between species from tropical and temperate areas respectively, as the latter tolerate lower temperatures compared with the former. Seed viability of intermediate seeds can be maintained for 9 months to 6 years under appropriate storage conditions (Hong and Ellis, 1996).

Examples of species with an intermediate seed storage behaviour are *Citrus* spp., *Chrysophyllum cainito* (Hong and Ellis, 1996) and *Coffea arabica* L. (Ellis *et al*., 1990).

Water sorption isotherms for intermediate seeds from several *Coffea*-species were intermediate between those shown by orthodox seeds and that of extremely desiccation-sensitive tissues, respectively. The former have a reverse sigmoidal, the latter a monotonic shape, according to investigations of Eira *et al*. (1999).

The properties of the seeds belonging to each storage category are summarised in Table 1.

Damage caused by desiccation of seeds

When water is withdrawn from seeds, three types of damage occur. When the cell volume is reduced during dehydration, mechanical damage may occur. This is important only in very hydrated tissues whose cells have large vacuoles. Metabolism-induced damage may be mediated by free radicals and by failure of protective antioxidant systems and cause aqueous-based degradative processes at intermediate water contents. Desiccation damage *sensu stricto* refers to removal of tightly bound water associated with the surfaces of macromolecules in cells, which leads to loss of structural integrity (Pammenter and Berjak, 1999).

Five states of water or hydration levels in seeds

Vertucci (1989) suggests that different levels of metabolism correspond to changes in the thermodynamic and motional properties of water, and that the transition from an anhydrotic state to a fully hydrated organism occurs in discrete stages. This is supported by Sun and Liang (2001), who found five discrete levels of critical water potential in desiccation sensitive seed tissues, and suggest that specific damaging and protective mechanisms exists at certain hydration levels.

As reviewed by Leprince *et al*. (1993), five states of water or hydration levels have been classified in orthodox and recalcitrant seed tissues by studying water sorption isotherms. In the first state, region one (0 to 8-10% (g H_2O/g DW)), the water molecules are very strongly associated with macromolecular surfaces by ionic bonding. In region 2 (8-22%), the interaction with macromolecules is weaker. At these levels, the water gains its ability to form glasses. At 22-33%, which is the third hydration level, water gains its solvent properties, and some metabolic activities are allowed. The properties appear to be that of

a concentrated solution. In region 4 (33-55%), the water exhibits similar properties as a dilute solution, and in region 5 (>55%), the tissues are considered to be fully hydrated, and seeds are able to germinate. Water properties in dried tissues appears to be of an obvious and primary importance in desiccation tolerance and important in determining survival during long-term storage of seeds.

Damage at the cellular level

Cellular membranes are primary sites of injury. When the membranes collapse, various cytoplasmic solutions leak out (Leprince *et al*., 1993). Injury at the membrane level may occur upon re-hydration of dry organs, while the cells remain essentially viable during the dehydration step (Hoekstra *et al*., 1989; Leprince *et al*., 1993). Drying changes the physical properties of the membrane phospholipids, so that they go from the liquid-crystalline phase to a gel phase at higher temperatures than at a hydrated state. T_m, defined as the temperature at which the lipid moves between a lamellar gel phase and a lamellar liquid-crystalline phase, increases. That is, a hydrated lipid that is in the liquid-crystalline phase at physiological temperatures will be in the gel phase at the same temperature if water is removed from the phospholipid headgroups. This is a reversible reaction. Formation of distinct gel-phase domains because of differences in phospholipidic composition, and thereby different phase transition temperatures, in the biological membrane, and phase separation of membrane lipids contributes to loss of membrane functions. These could be changes in permeability, compartmentation and membrane-bound enzyme activity, as the membrane and membrane-protein organisations are lost. It is also suggested that an oxidative attack that promote lipid peroxidation and/or phospholipid de-esterification, cause membrane desiccation injury. Membrane organisation and cellular compartmentation is lost. The source of free radicals may be impairment of respiration (Leprince *et al*., 1993).

According to Walters *et al*. (2001), at least two stress mechanisms are involved in desiccation damage of seed tissues. The predominant cause of viability loss in *Camelia sinensis* embryo axis was removal of water necessary for structural integrity (desiccation damage *sensu stricto*) if axes were dried to water potentials below -15 MPa and consumed less than 5000 μmol O_2/g dry mass during drying. At milder dehydration, the authors propose that a metabolic stress would be more likely to predominate. Pammenter *et al*. (1998) found indications of that the main cause of viability loss in slowly dried seeds from the recalcitrant species *Ekebergia capensis* Sparrm. was aqueous-based processes leading to membrane degradation. This suggestion was based on ultrastructural observations.

Pammenter and Berjak (1999) suggest in their review, that under natural conditions, the metabolism-induced damage at intermediate water contents is the cause of viability loss in recalcitrant seeds. These desiccation sensitive tissues are not able to switch off the metabolism, de-differentiate subcellular structures and they lack adequate protection against the consequences of dehydrating actively metabolising tissues.

Physiological mechanisms of desiccation tolerance in seeds

In their review, Pammenter and Berjak (1999) identified eight different physiological processes that may confer protection against injury caused by dehydration at different levels of water loss. These processes can be classified into the following:

a) intracellular physical characteristics, as reserve accumulation, DNA-conformation, vacuole volume reduction and protective molecule synthesis
b) intracellular de-differentiation of organelles that minimises the surface area of membranes
c) metabolic switch off
d) presence and operation of efficient antioxidant systems
e) accumulation of protective molecules, such as late embryogenic abundant proteins (LEAs), sucrose and oligosaccharids
f) amphipathic molecules that shelter the membrane lipids from phase transitions
g) an oleosin-layer around lipid bodies and
h) presence and operation of repair mechanisms during rehydration.

They suggest that the relative degree of desiccation sensitivity of seeds of individual species depend on the expression, or absence of one or more of these mechanisms. Leprince *et al*. (1993) concluded in the review of mechanisms of desiccation tolerance in developing seeds, that desiccation tolerance are not likely to be ascribed to one single mechanism. Rather, desiccation tolerance is a multifactorial property in which each component is equally critical.

Below, some of the mechanisms involved in desiccation tolerance will be described.

Antioxidant systems

In plant metabolism, free radicals are naturally produced, and plants must be endowed with antioxidant molecules and scavenging mechanisms, like enzymic systems that neutralise potentially toxic activated oxygen. Examples of antioxidant molecules are tocopherols, ascorbic acid and β-carotene, and the enzymes can be exemplified with superoxid dismutase, catalase, ascorbate and different peroxidases (Leprince *et al*., 1993).

Li and Sun (1999) found, that in recalcitrant *Theobroma cacao* seeds, decline of viability below the critical water contents was correlated with increased lipid peroxidation and cellular leakage. The activities of free radical-scavenging enzymes, such as ascorbate peroxidase, peroxidase and superoxid dismutase, decreased rapidly during desiccation below this water content. As the embryonic axis contained large amounts of sugars, raffinose and stachyose, desiccation-damage did not appear to be caused by lack of sugar-related protective mechanisms. More likely, damage was caused by decrease of the enzymic protection against desiccation-induced oxidative stress. According to the

authors, these results support a hypothesis that enzymic protection against free radicals plays an important role in desiccation sensitivity of recalcitrant tissues during desiccation.

Chaitanya and Naithani (1998) found that kinetin prolonged the viability of recalcitrant *Shorea robusta* Gaertn. f. seeds during storage at low (15°) temperatures. In kinetin-treated seeds, the superoxid-dismutase activity was discernable for longer periods, thereby reducing the oxygen free radical levels, leakage and lipid peroxidation during dehydration of tissues. According to the authors, kinetin appears applicable to enhance the storage potential for recalcitrant seeds.

Switching off of metabolism

At intermediate water contents (-11 to -3 MPa), metabolism is considered to be unregulated, and there is evidence of damaging reactions that probably are mediated by free radicals. Switching off metabolism is one way to avoid damage, as respiratory metabolism has the potential for free radical production under water stress. There are indications that recalcitrant seeds are metabolically active, which may be one reason for their desiccation sensitivity. Examples of mechanisms to switch off the metabolism are organelle de-differentiation, which reduces the membranous area vulnerable to free radical attack, and a decline in respiratory substrates (Pammenter and Berjak, 1999). Increased viscosity may also be regarded as a mechanism that controls the depression of metabolism during drying, as suggested by Leprince and Hoekstra (1998). They found that a rapid increase in viscosity during drying of soaked seeds of *Vigna unguiculata* and pollens of *Typha latifolia* and *Impatiens glandulifera* was accompanied by a decrease in respiration rates. The increase in viscosity may induce anoxia, which diminishes the danger of over-production of reactive oxygen radicals.

Sugars

As reviewed by Wolfe and Bryant (1999), non-specific solutes affect membranes at low or intermediate hydration. All solutes, such as salts and sugars, reduce the stress on membranes through their osmotic effect, but only if the solutes remain between the membranes. Otherwise, they can dehydrate membrane-rich phases and elevate the gel-fluid temperature, which leads to loss of membrane functions (see *damage caused by desiccation of seeds*). Different solutes have different effects, influenced by solute size and concentration. Desiccation-stress reduction on the membranes is only achieved if the solutes do not crystallise.

Pammenter and Berjak (1999) mention two hypotheses of the influence of non-reducing sugars on desiccation tolerance. The first of these, the 'water replacement hypothesis' suggests that specific sugars replace the water normally associated with membrane surfaces. By doing so, the lipid head-group spacing is maintained which prevents the damaging liquid- to gel phase-transition. The second is that the aqueous phase vitrifies, leading to a 'glassy state'. At dehydration, sucrose and certain oligosaccharides form high-

viscosity, amorphous, super-saturated solutions, which slow molecular diffusion, thereby slowing down chemical reactions. This reduces the deleterious effect of deranged metabolism. It may also prevent or reduce phase transitions in the membrane bilayer (Pammenter and Bejrak, 1999). Leopold *et al*. (1994) conclude that the glassy state may contribute to protection of membrane integrity during desiccation, but it does not appear to account for desiccation tolerance. In dry seeds, the major role of the glassy state is to serve as a physical stabiliser and thereby contribute to the stability of the seed components during storage.

Sucrose does not seem to be a key determinant of desiccation tolerance (Lin *et al.*, 1998). By hydration and re-hydration studies on seeds of five species of crop (okra, cucumber, tomato, mung bean and snow pea) this conclusion was drawn since monosaccharide content increased slightly or were maintained at a constant level before the seeds lost their desiccation tolerance. As oligosaccharide content decreased to a low level, the desiccation tolerance disappeared. Even if sucrose levels were two or three times higher than original levels at the time of losing desiccation tolerance in snow pea and mung bean, no prevention of desiccation damage was seen. In cucumber, the sucrose content was higher in the more desiccation sensitive radicles than in the hypocotyl. In orthodox *Pisum sativum* L. seeds, the (raffinose+sucrose):sucrose ratio increased during seed development (Corbineau *et al.*, 2000). Acquisition of desiccation tolerance in the fresh seeds was associated with an accumulation of raffinose and stachyose. In immature seeds, extracted from the pod, no stachyose was synthesised, and neither did the seeds tolerated fast drying. That treatment resulted in electrolyte leakage and metabolic dysfunction. In contrast, increased oligosaccharide content, resulting in a decrease in sucrose content, was seed when immature seeds were dried in the pod or at high relative humidities. In this case, the seeds germinated and produced a normal seedling, and therefore considered as tolerant. However, in ten *Coffea*-species, desiccation sensitivity was not significantly related to the sugar content, nor the presence/absence of oligosaccharides (Chabrillange *et al*., 2000).

Steadman *et al*. (1996) investigated the possibility that identification of seed storage behaviour could be based on sugar analysis. They studied the carbohydrate composition of 46 tissues from 18 species, and found that in general, orthodox and recalcitrant seed tissues have sucrosyl-oligosaccharide:sucrose mass ratios of >0.143 (1:7) and <0.083 (1:12), respectively. However, large variations in the content of these sugars within the recalcitrant and intermediate seeds were noted. In 17 of 18 dissected seed lots, the axis contained more soluble sugars than the cotyledons. The authors suggests that analysis of oligosaccharide:sucrose ratios could be a useful first step in rapid screening of germplasm for predicting storage behaviour, but as there are exceptions to the "general rule" mentioned above, the reliability of a single technique, such as sugar analysis, are limited. A particular example of this is the study of Chabrillange *et al*. (2000). They found that in *Coffea*-species, the oligosaccharid:sucrose ratio was not related to seed desiccation sensitivity.

As reviewed by Leprince *et al*. (1993), sugars may also stabilise protein structures during desiccation. At least in desiccation tolerant dry onion seeds, the secondary structures of

proteins were very stable, even after decades of storage and loss of viability. Whether this could be ascribed to stabilisation by sugars is not evident from the study (Golovina *et al*., 1997).

Trehalose, the most effective stabilising sugar (Crowe *et al*., 1987) has not been detected in seeds (Leprince *et al*., 1993). However, other soluble sugars are present. Disaccharides are in general superior to other sugars as a protective agent, but there is considerable variability among the disaccharides. Comparative studies on the stabilising effect of sugars have shown that trehalose was the most effective. Trehalose was followed by a group of disaccharides, including maltose, raffinose and sucrose, then monosaccarides. Inositol was the least effective agent (Crowe *et al*., 1987).

Amphipathic molecules

Hoekstra *et al*. (1997) suggests that although sugars play a role in desiccation tolerance, they may share it with amphipathic molecules where it concerns prevention of membrane dysfunction by depressing the phase transition temperature constant (T_m). These amphipathic molecules partitions into the membrane when it is undissociated or highly concentrated at low water contents, and thereby interacts with membrane phospholipids. According to Pammenter and Berjak (1999), it is a reversible reaction, and it may account for leakage when the dry, desiccation tolerant tissues are re-hydrated. It is possible that the necessary amphipaths are absent or non-functional in desiccation sensitive tissues.

Buitink *et al*. (2000) observed a relationship between desiccation tolerance and the transfer of amphiphilic molecules from the cytoplasm into lipids during drying of imbibed radicles of *Pisum sativum* and *Cucumis sativa* seeds. Desiccation tolerance was found to be present during early imbibition, but was lost in germinated radicles. By studies using paramagnetic resonance spectroscopy of amphiphilic spin probes, they found that partitioning of spin probes into lipids during dehydration occurred at higher water contents in desiccation sensitive radicles than in tolerant, a difference that could not be explained by water loss. A transfer process at higher water contents is more likely to induce metabolic dysfunction due to disturbances in membrane structures than if partitioning occurs at lower hydration levels, where, in effect, the long-term stabilisation of membranes in the dried state could be improved. A rise in cellular microviscosity was seen at higher water contents during drying of desiccation sensitive tissues compared with tolerant. This suggests that the microviscosity of the cytoplasm control the transfer process of amphiphilic compounds into lipids of tissues in dehydrating seeds (Buitink *et al*., 2000).

Oleosins

In seeds, one of the major storage compounds is fats/oils, the most efficient form of energy storage. These oils are stored as liquid triacidglycerols in small, discrete subcellular droplets, called oil bodies, and are mobilised during germination and seedling

establishment. Storage in small oil bodies provides an increased surface for activity of metabolic enzymes, lipases, when the demand of energy increases. Storage of water-insoluble triacylglycerols in discrete particles that not coalesce inside the cell requires special mechanisms for stabilising the oil bodies, especially their surfaces. This protection is provided by oleosins. Oleosins are alkaline proteins of low molecular mass and are unique to the oil bodies. They are embedded in a monolayer of phospholipids that surrounds the triacidglycerols in the oil bodies. Together with the phospholipids, they stabilise the oil bodies and maintain the organelles as small entities via their surface charges and steric hindrance. They are also suggested to provide specific binding sides for lipase during germination, or act as a lipase activator (Huang, 1992).

Leprince *et al*. (1998) reports that oil bodies in recalcitrant oilseeds (*Theobroma cacao* L. and *Quercus rubra* L.) contain only small amounts or no putative oleosins. In orthodox *Sterculia setigera* Del. and intermediate *Coffea arabica* L. seeds, the ratio of oleosin:oil was similar to that in rapeseeds. During drying, the oil bodies remained stable in all of the species studied, regardless of oleosin content. However, at rehydration of artificially dried seeds containing large amounts of storage oil bodies but little or no oleosins, the oil bodies showed a strong tendency to coalesce. This indicated that oleosins might have a stabilising role during imbibition prior to germination. The oleosins may indirectly contribute to desiccation tolerance, because any dehydration test must incorporate a rehydration phase. The authors also suggest that during seed imbibition, water flow into cells containing large numbers of oil bodies not protected by oleosins, will result in coalescence in order to reduce the interfacial tension of the oil-water interface. The coalescence disrupts the cellular structure, which was apparent in electromagnetic microscopy of rehydrated cocoa cotyledons. Therefore, desiccation damage in oil-rich recalcitrant and intermediate seeds could be a consequence of deficiencies in their oleosins (Leprince *et al*., 1998).

Factors that affects the desiccation tolerance of seeds

Drying rate

It has been found that the more rapidly dehydration can be achieved in desiccation sensitive seeds or tissues, the lower the water content to which the seeds or embryo-axes can be dried before viability is lost, especially when drying excised axes (Farrant *et al*., 1993, Pammenter *et al*., 1998, Pammenter and Berjak, 1999). As reviewed by Pammenter and Berjak (1999), a suggested explanation is that a rapid dehydration shortens the period during which aqueous-based deleterious processes at intermediate water contents can occur. The desiccation sensitivity of recalcitrant seed tissues depends partly on the metabolic activity of the tissues: very rapid dehydration minimises the damage associated with dehydration of metabolically active tissues. However, the lower limit to which a recalcitrant tissue can be dried, is always higher than the water content to which intermediate and orthodox tissues can be dried. The influence of drying rate is much less marked for whole seeds than for axes, probably because whole seeds are too large to be dried sufficiently rapid for the effect to become pronounced. It is also possible that

dehydration inside a seed or an organ is uneven during rapid drying. This can result in that the tissues essential for germination (axis meristems) are actually at higher water contents than are measured for the whole seed or axis.

Farrant *et al*. (1993) showed that excised embryo axes of the recalcitrant species *Avicennia marina* Forssk. survived at lower water contents if rapidly dried than following slow drying. The same was true for recalcitrant seeds of *Ekebergia capensis* Sparrm. (Pammenter *et al*., 1998). Rapid drying of whole seeds decreased the water contents below which viability was lost. Slow drying brought about homogenous dehydration whereas rapid drying was uneven across the seed tissues, as seen by the time course of decline of axis water content. This uneven dehydration during rapid drying could not be rejected as a contributory reason for the better survival of rapidly dried seeds. The authors suggest that because of the influence of drying rate on desiccation tolerance, it is not possible to determine critical water contents for recalcitrant seeds. In contrast, Liang and Sun (2000) claimed that there is an optimal drying rate, with which the maximum desiccation tolerance of a recalcitrant seed can be achieved. The optimal drying rate is related to a minimal level of combined damages from mechanical and metabolic stresses during dehydration. By using the equilibrium dehydration method, this optimal drying rate could be determined, and the critical water potential, corresponding to maximum desiccation tolerance, can be evaluated.

In developing orthodox seeds and desiccation tolerant vegetative tissues, better survival of dehydration accompanies slow drying, presumably because sufficient time is allowed for induction and operation of protective mechanisms (Pammenter and Berjak, 1999).

Drying rate can also influence when under maturation a seed gains its desiccation tolerance. Two patterns are observed. Either, desiccation tolerance is gained coincidentally with the ability to germinate, or before it. When entire seeds were dried slowly, they gained desiccation tolerance coincidentally with germination capacity, but when they were rapidly dried, they gained it earlier (Leprince *et al*., 1993).

Tissue

Studies have shown varieties between desiccation tolerance in different recalcitrant seed tissues (Lin *et al*., 1998; Pammenter *et al*., 1998; Li and Sun, 1999; Pammenter and Berjak, 1999). This can be exemplified with *Theobroma cacao* cotyledons, which tolerated desiccation to lower water contents than embryonic axis of the same species (Li and Sun, 1999) and radicles of cucumber were far more sensitive to desiccation than hypocotyls in imbibed seeds (Lin *et al*, 1998). In general, embryonic axes of desiccation sensitive species are more tolerant to dehydration when they are isolated than if dried in the whole seed (Kermode and Finch-Savage, 2002).

Degree of development at harvest

During development, an orthodox seed progress through phases of histodifferentiation, where there is an increase in fresh mass; reserve deposition, accompanied by a rapid increase in dry mass, and maturation drying, where dry mass accumulation ceases and fresh mass decreases as the seed loses water. In recalcitrant seeds, the histodifferentiation is similar to that in orthodox seeds. This phase is followed by reserve accumulation, but in contrast to orthodox seeds, dry mass continue to accumulate until the seeds are shed and no maturation drying occur. However, in all but one species (*Avicennia marina*) investigated so far, there is a decrease in water content at the end of development, which results from faster accumulation of dry matter than of water. In most recalcitrant seeds, desiccation tolerance increases as water content is diminished towards the end of development (Pammenter and Berjak, 1999).

Ellis *et al*. (1987) studied the development of desiccation tolerance in six orthodox grain legumes (*Vicia faba* L., *Lens culinaris* Medic., *Cicer arietinum* L., *Lupinus albus* L., *Glycine max* L. and *Pisum sativum* L.). Physiological maturity, that is, when seeds reach their maximum dry weight during development, occurred when maturation drying on the parent plant had reduced seed moisture content to approximately 60%. This coincided with the onset of desiccation tolerance measured by the ability of seeds to germinate following harvest and rapid artificial drying, except in pea, where desiccation tolerance was acquired a little earlier, at 70% water content. Harvest beyond the optimal moisture content at harvest reduced viability and increased the frequency of seedling abnormalities in *V. faba*, *G. max* and *P. sativum*. Golovina *et al*. (2001) found that in *Triticum aestivum* L., embryos were able to germinate after rapid drying and rehydration after completion of morphological development, which is a few days before mass maturity (physiological maturity). They also observed that drying of premature kernels on the ear of plants cut earlier than five days after anthesis were unable to germinate, but cellular desiccation tolerance was nevertheless acquired, which lead to the conclusion that desiccation tolerance is independent of seed morphological development. However, according to Hong and Ellis (1996), many orthodox species show damage upon desiccation to very low moisture content levels at later stages of seed development, such as the end of the seed filling phase.

In studies on the recalcitrant species *Quercus robur* L., Finch-Savage (1992) found that the onset of a reduction in sensitivity to desiccation during development on the tree, coincided with the acquisition of seed germination capacity. Tolerance to desiccation increased throughout development to shedding, but viability was lost at relatively high moisture contents, and therefore, the seeds never passed through a fully desiccation tolerant phase. This suggests that desiccation sensitivity in *Q. robur*, and probably also in other recalcitrant seeds, may have resulted from early termination of development. Thus desiccation tolerance was never achieved but rather lost because of the onset of germination. Studies on *Avicennia marina* Forssk. demonstrated that prior to the acquisition of full germinability, which was midway through the phase of growth and reserve accumulation, the seeds were unable to tolerate any dehydration. Thereafter, they became tolerant to slight water loss, and desiccation tolerance was not influenced by the

stage of development (Farrant *et al*., 1993). Another example of differences in desiccation sensitive tissues comes from a study by Li and Sun (1999). They found that mature and immature axes of *Theobroma cacao* seeds tolerated desiccation to critical water contents of 1,0 and 1,7 g/g DW, respectively, under a rapid drying regime. In *Coffea canephora* Pierre ex Froehner, seeds extracted from ripe or almost ripe fruits were less sensitive to loss in viability from desiccation than those from immature fruits (Hong and Ellis, 1995). This is in accordance with other studies (Pammenter and Bejrak, 1999) and suggests that pre-harvest activities reduce the desiccation tolerance of recalcitrant seeds and desiccation sensitive tissues.

Environmental influence

The environmental conditions during seed development on the mother plant can have an influence on the desiccation tolerance after maturation and shedding. Dussert *et al*. (2000) investigated the relationship between desiccation sensitivity, seed water content at maturity and climatic characteristics of native environments of nine *Coffea* L. species. They used previously reported data on desiccation tolerance, data from nine climatic stations and continuous sequences of rainfall data. They found that the mean number of dry months that seeds had to withstand after shedding was significantly correlated with parameters used to quantify seed desiccation sensitivity, namely water content and water activity at which half of the initial viability was lost. Thus, the authors suggest that the higher level of tolerance to desiccation corresponds to an adaptation to drought. However, there was no correlation between duration of seed development and seed desiccation tolerance, neither between seed moisture content at maturity and the level of desiccation tolerance.

In *Aesculus hippocastanum* L. seeds, variations in germination rates were found between seed lots from two different years (Tompsett and Pritchard, 1998). The authors suggest that such inter-seasonal differences may be temperature-related.

Handling and storage

In fully germinable seeds from *Avicienna marina* Forssk. storage lifespan under non-desiccating conditions was considerable reduced by the presence of the pericarp, probably because of fungal contamination (Farrant *et al*., 1993). In IPGRIs protocol to determine seed storage behaviour, several factors that may influence desiccation tolerance are pointed out. Among these are extraction of seeds, which can mechanically damage the seeds, packing during transport and temporary storage conditions (Hong and Ellis, 1996). Seed germination takes place in three successive phases: imbibition (water intake, increase in respiratory activity), germination *sensu stricto* (activation of the embryo, no morphological changes) and growth (beginning of radicle elongation) (Côme and Corbineau, 1989). After the onset of imbibition, the desiccation tolerance is maintained for several hours. Prior to radicle emergence, seeds can withstand extreme drying, but as germination progress, such treatment becomes highly damaging,

sometimes lethal (Leprince *et al*., 1993). Therefore, any method involving long exposure to high seed moisture content at temperatures at which some progress towards germination is possible can increase desiccation sensitivity (Hong and Ellis, 1996).

If the desiccation tolerance test will be performed and evaluated through germination tests, the storage conditions before the test start are of importance. Pammenter *et al*. (1998) found that short-term, rapid drying of recalcitrant seeds of *Ekebergia capensis* Sparrm. increased the rate of germination after re-hydration. Partial drying followed by chilling of naturally shed *Aesculus hippocastanum* L. seeds increased germination percentage and rate (Tompsett and Pritchard, 1998). Further drying was followed by a detrimental effect. The partial desiccation was not essential for completion of developmental processes in the seeds. Futhermore, seeds are sometimes dormant and will not germinate until the dormancy is broken, for example by a period of chilling (Baskin and Baskin, 1998).

Some ecological aspects of seed storage behaviour and desiccation tolerance

Plant ecology

Yeheb, *Cordeauxia edulis* Hemsl., is native to semi-arid and arid regions in Ethiopia and Somalia, where annual rainfall do not exceed 400 mm. According to Hong and Ellis (1996), seeds with recalcitrant storage behaviour do not occur naturally in such areas, while a few species may show intermediate and the majority orthodox seed storage behaviour. There are, however, exceptions. Danthu *et al*. (2000) found that the African tree species *Boscia senegalensis* (Pers.) Lam. ex Poir. from the Sahelian zone, where annual rainfall is 100-600 mm, could be classified as recalcitrant. Viability in the seeds was totally lost when moisture content dropped below 18% and temperatures close to zero elicited symptoms of chilling injury. In wet storage at 15°C, the seeds were viable for two months. According to Hong and Ellis (1996), further generalisations of association between seed storage behaviour and plant ecology is impossible.

Moisture content at shedding or maturity and seed size

At maturity or shedding, species with recalcitrant seed storage behaviour contains 36-90% water, intermediate 23-55% and orthodox <20-50% (wet weight basis). There is also an association between seed size and storage behaviour. Seeds with a thousand seed weight (TSW)>13 000 g are not likely to be orthodox, and small seeds, TSW<25 g, are mostly orthodox, although there are exceptions. Seeds with TSW 30-13 000 g may show orthodox, recalcitrant or intermediate storage behaviour (Hong and Ellis, 1996).

Taxonomy and interspecific variations

According to Hong and Ellis (1996), species in Chenopodiaceae, Combretaceae, Apiaceae, Lamiaceae, Solanaceae and Pinaceae are generally orthodox, while species in Rhizophoraceae are recalcitrant. Species within Fabaceae, Poaceae, Cucurbitaceae, Brassicaceae and Rosaceae show orthodox seed storage behaviour, but there are several notable exceptions. Yeheb belongs to Fabaceae, tribe Caesalpiniaceae, (Bally, 1966), the same as the recalcitrant African tree *Cordyla pinnata* Lepr. (ex. Rich) M.-Redh. The latter species grows in Sudan, annual rainfall 400-1500 mm, and has seed moisture contents at shedding of 52%. *C. pinnata* lost seed viability rapidly when seed moisture contents were <30%, and did not germinate at 24% (Danthu, *et al*., 2000). However, seed storage behaviour, and thus, desiccation tolerance, can differ among species within a genus (Hong and Ellis, 1996). Among *Coffea* spp., *C. arabica* L. and *C. canephora* Pierre ex Froehner are classified as intermediate, while *C. liberica* Bull ex Hiern is recalcitrant, but appears to be rather less sensitive to desiccation than certain other recalcitrant species (minimally recalcitrant). *C. arabica* seemed to be more desiccation tolerant than *C. canephora*. These variations may be the result of an association with plant ecology, where the desiccation tolerant species origin from dryer regions, whereas the less tolerant species are adapted to more humid climates. Also, in *Citrus*, there is evidence to suggest variations in seed storage behaviour. In this case, the species are either orthodox or intermediate (Hong and Ellis, 1995) and there are differences in the degree of desiccation tolerance among the species in the same category (Hong and Ellis, 1995; Saipari *et al*., 1998).

Desiccation experiment with yeheb (*Cordeauxia edulis* Hemsl.) seeds

Introduction

Are the seeds from yeheb (Cordeauxia edulis Hemsl.) recalcitrant, intermediate or orthodox? Johnson (1996) reports yeheb seeds to show recalcitrant storage behaviour, but no scientific publications are available. The seed moisture content of yeheb seed at shedding was unknown at the beginning of the study, and the previously reported thousand seed weight (TSW) was 2000-3000 g (Nerd, 1994). According to Hong and Ellis (1996), seeds with TSW>13 000 g are not likely orthodox, while small seeds, TSW<25 g, are mostly orthodox. At maturity, recalcitrant seed contains 36-90% water, intermediate 23-55% and orthodox <20-50% (wet weight basis). Thus, MC and TSW of yeheb seeds give no indication to the storage category of yeheb seeds.

According to IPGRIs revised desiccation and storage protocol, 11.11. (IPGRI, 2000), seeds will be considered recalcitrant if "the relationship between moisture content and germination capacity reveals a critical moisture content at 15-20% or higher". If the seeds do not lose viability when dried to about 5% moisture content or below (wet weight basis), they are likely to be orthodox. If most or all seeds tolerate dehydration to 10-12.5% moisture content, but further desiccation reduces viability, the seeds are probably intermediate (Hong and Ellis, 1996). A simplified outline of the procedure is shown in Fig.1. If the seeds are orthodox or intermediate, further research of the seed behaviour in storage is necessary to determine the classification, as orthodox seeds can be stored for several years in sub-zero temperature, while intermediate seeds lose viability if stored too cold (Hong and Ellis, 1996). The main objective with this study was to investigate the desiccation tolerance of "newly" harvested yeheb seeds.

Figure 1. Simplified outline of determination of seed storage category. Adapted from Hong, T.D. and Ellis, R.H. (1996).

Materials and methods

Collection of seeds and transport to Sweden

Yeheb seeds used in this experiment were collected from 1 ha of land near the village Bokh (ca 7.5°N, 47°E), in the Ogaden region in eastern Ethiopia. This year, 2003, the first of the two rainy seasons began on 28 April and ended on 24 May. The total rainfall received was 101 mm at 5 occasions. The relative humidity and temperature varied during the day, from 43-72% and 28-36°C, respectively (registered at 7.20 to 15.10, on 20 May to 26 May). People started harvesting the fruits on 21 May, while the pods were

still green and had not reached full maturity. For our experiments only the pods shed from the mother shrubs, and thus deemed to have attained full maturity, were used. The first pods, which when maturing change colour from green to dark brown, were shed on 24 May. More pods were then shed and collected in the period of 26-29 May (A. Yahya, *pers. comm.*).

After collection, 104 representative pods, seeds and pericarps were weighed. The average weight of the pods was 7.95±2.49 g, the thousand seed weight 5040 g and the average weight of the pericarps 2.91±0.95 g.

Since it was not known whether yeheb seeds could keep their viability at low moisture contents, the fruits were transported in order to minimise water loss. The fruits were transported in loosely sealed plastic bags, allowing passage of air. Cotton was placed in the plastic bags to absorb excess moisture from the fruits, which might favour germination. The plastic bags were then packed in cardboard boxes. The minimum and maximum temperatures were monitored during the transport. At the arrival of fruits in the laboratory, it could be confirmed that the fruits had not been subjected to extreme temperature in the cardboard, since the minimum and maximum temperatures were +18°C and +45°C, respectively (A. Yahya, *pers. comm.*).

Condition of seeds and determination of initial moisture content at the arrival

The seeds I used in my study were similar to paranuts in size and shape. The smallest seeds, which were found in pods containing more than one seed (not more than two in each pod for the fruits I used. It was an uncommon feature.), were peanut-sized, or in some cases, hazelnut in shape and size. The fresh seed had a sweet, nutty, but "grassy" taste, the roasted tasted like a mixture of roasted chestnut and cashew. The thousand seed weight was 3100-4500 g, based on samples of 25 seeds. Based on one sample of 104 seeds weighed in the field, the thousand seed weight was 5040 g.

About 15% of the seeds germinated before reaching the laboratory, on 7 June, 2003, 10-13 days after harvest. Most of the fruits and many seeds were epiphytically infected by fungus.

Fresh weight and initial moisture content (MC) was determined on each of 20 seed lots. Four seeds from each seed lot were weighed, cut into small pieces, dried at 103°C for 17 hours and weighed again. Moisture content was 41.6% in average (35.7-47.4%, standard error 3.44) on a fresh weight basis. When calculating the TMC (target moisture content) for each replicate to the desiccation test, a MC value of 40% was used.

Preparation of seeds

Mature pods (fruits) of yeheb, the different seed lots mixed, were peeled on 7-8 June, 2003. Only seeds without mechanical injury were used, but some were epiphytically

infected by fungus. The seeds were soaked in 1% sodium hypochlorite solution for five minutes followed by five minutes in de-ionised water. The seeds were blotted dry carefully between two sheets of filter paper and placed in unsealed romb-shaped glass containers (2000 ml; IKEA, Sweden) until divided into portions of 12 seeds for the initial germination test with fresh seeds and portions of 25 seeds for the desiccation tolerance test. The average seed weight was 97.41 g/portion (TSW 3100-4490 g). Seeds to be used in the desiccation tolerance test were placed in open Erlenmeyer flasks (500 ml) for one day in room temperature before the desiccation of the seeds started (9 June).

Desiccation of seeds

Twenty-five seeds were mixed with 98 g (135 ml) of silica gel in each of 24 IKEA-containers, giving 4 replicates for each of six target moisture contents (TMC) (see Table 2). To test the effect of dehydration time on germination, 25 seeds were mixed with 135 ml vermiculite in each of three IKEA glass containers. This treatment was terminated when the lowest TMC was achieved. There were only three replicates of the time control, because of insufficient seed supply. Containers were placed in room temperature. The silica gel was changed in all containers at the same time when the gel was saturated with water. This was indicated by a change in colour from blue to pink. At the same time, the vermiculite in the control was changed. During daytime, the containers were carefully shaken regularly, ca. once every hour.

Water loss were monitored by weighing the seeds regularly (silica gel removed by sieving), more frequently at the beginning of the desiccation and near the calculated TMC. During the night, desiccation was interrupted if necessary, depending on desiccation rate monitored by the above described weighing and on TMC. The seeds were then stored in sealed IKEA-containers, without silica gel until drying was resumed.

Decision of the different TMCs were based on the recommendations from IPGRIs revised desiccation and storage protocol (2000), for seeds with initial MC between 35 and 40% on a wet weight basis. TMC was calculated by using the formula:

$$\text{Weight of seeds (g) at TMC} = \frac{100 - \text{MC at start of desiccation}}{100 - \text{TMC}} \times \text{initial seed weight(g)}$$

When calculation showed that TMC was reached, desiccation was terminated (3-97 hours). Five seeds from every container was used for moisture content determination, the other 20 for a germination test according to methods described below. Some seeds (<4 seeds/replicate) showed signs of mechanical injury after desiccation in silica gel. These seeds were used for moisture content determination, so that all seeds in the germination test appeared to be in good condition, except a superficial layer of fungus on the testa. Aimed and attained target moisture contents are shown in Table 2. Seeds with TMC <15% were humidified to avoid imbibition damage before the germination test started. This was done over a water surface in sealed IKEA-containers (2000 ml) until seed weight had increased by 12.6-14.3% (4-5 days). Ca. 200 ml de-ionised water was used in

each container. A perforated plastic shelf was placed above the water surface in each romb-shaped container. On this, four net rings, ca. 6 cm in diameter were placed. Each net ring contained five seeds, so that all 20 seeds from each replicate were humidified in the same container at the same time.

For determination of moisture content, the seeds were weighed, cut into small pieces and dried for 17 hours in 103°C and weighed again. Moisture content (MC) was calculated by the formula:

$$\text{MC}\,(\%) = \frac{\text{Weight of seeds (g) after desiccation} - \text{dry weight of seeds (g)}}{\text{Weight of seeds (g) after desiccation}} \times 100$$

Table 2. Aimed TMC and attained MC after desiccation in silica gel/vermiculite and duration of drying

Aim TMC (%)	5	10	20	25	30	35	Control
Attained MC (%)	9.6	12.3	24.4	27.4	33.4	39.3	40.1
Standard error	4.7	2.2	1.3	2.5	1.0	2.4	1.8
Duration of drying (h)	81.4-97.1	57.9-82.3	19.3-22.8	15.1-16.5	9.1-9.2	3.2-3.6	97.1

Germination test

For the initial germination test with fresh seeds, 8x12 seeds were placed in plastic dishes immediately after the seeds had arrived to Sweden, at 7 June, 2003. For the germination test with desiccated seeds, each replicate with 20 seeds was divided among two dishes. Each dish (16 cm bottom and 18 cm top diameter and 3.6 cm deep) was filled with 660 ml sterilised sand (12 h, 105°C), wetted with 200 ml de-ionised water and covered with a plastic bag. Seeds were laid on the lateral side so that only half of the seed was covered by soil. The dishes had been prepared 1-7 days before the germination test began and stored in room temperature until seeds were sown. The germination test was initiated immediately when the seeds had reached the calculated TMC (9-11 June) or when seeds with TMC <15% had been humidified (17 June). For the control treatment, the germination test started at the same time as the last desiccation treatment of seeds was placed in a container for humidifying (13 June).

The germination test was performed in an incubator with a diurnal temperature and light regime of 12/12 h at 25/15°C in light/darkness, photon irradiance 50 μmol $m^{-2}s^{-1}$. The light period coincided with the high temperature. The dishes were piled two and two upon each other, due to lack of space in the incubator. They were shifted twice daily during the first two days of germination (after three and nine hours in light), then once every day. The first two days of the germination test of each TMC, the dishes was placed on the upper shelf, thereafter, they were shifted between the upper and the lower shelf once or twice weekly. The plastic bag was changed when, and if, too wet and dirty because of fungal contamination from infected seeds, and the dishes were watered if the sand was

dry (50 ml de-ionised water). Germination assessment was conducted at least twice every week. Seeds were considered germinated when the radicle appeared and was about 3 mm.

The germination test was terminated after four weeks (4-15 July, depending on TMC). Seed viability was assessed by finger pressing upon the seeds. Seeds that were soft or spongy were considered as dead. Seeds that were firm, possibly viable, were rinsed in de-ionised water and left to dry for three days in open dishes in room temperature. They were then placed in dishes for a further germination test with gibberellic acid and potassium nitrate (described below). At termination of this second germination test viability of non-germinated seeds was first assessed by finger pressing upon the seeds, then, if the seeds were firm, by using the tetrazolium test (described below).

Germination test with gibberellic acid and potassium nitrate

Treatment with gibberellic acid and potassium nitrate are recommended as germination promoting and physiological dormancy breaking methods (International Seed Testing Association, 1985).

The non-germinated, firm and "dry" seeds were placed in plastic dishes (size as in the germination test), each dish filled with 330 ml sterilised sand wetted with 100 ml gibberellic acid/potassium nitrate solution (0.5 g and 2 g/l de-ionised water, respectively) and covered with a plastic bag (7-18 July). The seeds were again laid on the lateral side so only half of them were covered by soil. The dishes were placed in the incubator (same temperature and light regime as described above) for two weeks of further germination. This time, the dishes were not piled upon each other and they were only placed on the upper shelf in the incubator. Germination assessment was conducted twice a week. After 13-14 days of germination, the test was terminated (21-31 July).

Tetrazolium-test for viability

2,3,5-triphenyltetrazolium chloride (tetrazolium) is a colourless, water-soluble salt that is able to pentetrate imbibed seed embryos. The salt is reduced to a red, insoluble formazan by dehydrogenases, which are only active in viable cells. The formazan is trapped within these cells, and since it is red, the tissues that contain viable cells can be identified. However, the tetrazolium test is not an absolute test of seed viability and shall be regarded as an estimate of the seed viability (Ellis *et al*., 1985).

In 400 ml de-ionised water, 3.631 g KH_2PO_4 was dissolved. In another flask, 7.126 g $Na_2HPO_4x2\ H_2O$ was dissolved in 600 ml de-ionised water. The two solutions were mixed to a buffer solution. 10 g of 2,3,5-triphenyl tetrazolium chloride was dissolved in 1000 ml of the buffer solution, which gave a concentration of 1%. The non-germinated, possible viable yeheb seeds were cut transversally, so that the cotyledons were split in half. As the seed coat (testa) of yeheb seeds may contain the red pigment cordeauxiaquinon and to assure rapid and even staining, it was removed, the seed kept

moist until the whole replicate was prepared. The seeds were then soaked in tetrazolium solution at 30-33°C in darkness for 24 hours. The seeds were completely covered by the solution. After the staining treatment, the seeds were washed several times in de-ionised water to remove any excess tetrazolium-solution. The seeds were evaluated immediately by splitting the two cotyledons, exposing the radicle. The cotyledons were also split longitudinally to facilitate evaluation of the staining pattern. If the cotyledons and the radicle were evenly stained in a bright, red colour (>80% of the seed), the seed was considered as viable.

Statistical analyses

Differences in germination percentage between different TMC, were analysed using the GENMOD procedure (SAS Institute Inc., 1999). A logit analysis was performed, assuming binomial distributed data and a logit link function. Seeds from the two dishes of each replicate from the desiccation treatment were considered as one value, so that in total 27 values from the desiccation treatment were used. To balance the number of seeds in the germination test with fresh seeds, with the number of seeds in each replicate of the desiccation-tolerance test, the eight dishes from the former test were fused two and two, giving 4 values (replicates) from the germination test with fresh seeds. Had each dish been considered individually, the results of the statistical analyses may erroneously have shown significant differences between the treatments because of an increased number of samples, thereby increasing the number of degrees of freedom.

In a first step the effect of MC on the germination was tested. Also, pairwise comparisons were conducted between TMC 5 and 10, TMC 10 and 20, and between TMC 35 and the fresh seed control. To test the effect of time during dehydration, the difference in germination between fresh seeds and seeds kept in vermiculite (time control) was analysed.

Since no calibration of the tetrazolium test of viability of yeheb against fresh seeds had been performed, results of the test were considered uncertain and not analysed statistically.

Results

When the first four weeks of germination was ended, 70-84% of the seeds with moisture contents >24% had germinated (Fig.2). Germination percentage was 58% and 41% for MC 12,3% and 9,6%, respectively. Most of the seeds that had not germinated were soft and spongy when pressing a finger upon them, and consequently considered as dead. Many of them were heavily infected by different species of fungus. Most seeds (>90% of those that germinated in each replicate) had germinated within eight days from sowing, and no seeds germinated later than 15 days after they were sown.

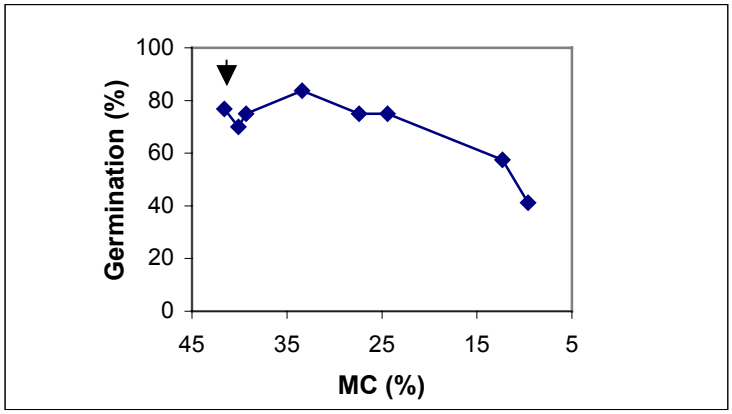

Figure 2. Germination (%) of fresh *C. edulis* seeds, after dehydration in silica gel for 3-97 hours. The fresh seed control is marked with an arrow.

No seeds germinated during the gibberellic acid and potassium nitrate treatment. All seeds with moisture contents >24% were dead when this germination test was interrupted. They where all soft and spongy because of fungal attack. Of 17 and 11 seeds with TMC 5 and 10%, respectively (moisture contents 9.6 and 12.3%), seven seeds from each TMC, were still firm when ending the germination test with gibberellic acid and potassium nitrate. The tetrazolium test indicated that two seeds of the former, and one of the latter moisture contents may be viable as the red staining of the seeds was >80%. However, there were traces of fungal infection also on these seeds, and the staining pattern appeared somewhat patchy. At least on one of the seeds (MC 12.3%), the radicle seemed injured, seen as a grey/brown coating at the tip.

The statistical analysis showed that there was no significant influence of the time factor (fresh seeds compared to vermiculite, p-value 0.3456 according to the LR-statistics). However, the proportion of germinated seeds was dependent on seed moisture content (p-value 0.0001). Pairwise comparisons showed that seeds at 9.6% and 12.3% MC germinated significantly less than fresh seeds (p-value 0.0001 and 0.0062, respectively). In addition, there were significant reductions in germination percentage between 24.4 and 12.3% and between 12.3 and 9.6% MC (p-value 0.0187 and 0.0394, respectively).

Discussion

In this study, the seeds were dried in silica gel to different "target moisture contents" (TMC) based on the initial moisture content of fresh seeds. Desiccation tolerance was assessed by germination tests. The seeds were germinated for four weeks in moistened sand, followed by two weeks with gibberellic acid and potassium nitrate added to the substrate. The viability of non-germinated seeds was evaluated by the tetrazolium test.

Germination and seed storage behaviour

Germination of yeheb seeds was strongly dependent on seed moisture content. The seeds did not seem to be recalcitrant, as they were still able to germinate after desiccation to <12.3% moisture content. However, germination percentage was reduced when the seeds were dried to the two lowest moisture contents. Unfortunately, attempts of drying of seeds to 5% moisture content or below failed. Also, there was no period of storage prior to the germination and viability tests in this study, which makes it difficult to classify the seeds as orthodox or intermediate without further experiments. Probably, the seeds are intermediate since they germinated, although at reduced germination percentage, at moisture contents of 9.6 and 12.3%. According to previous reports, the seeds lose viability in a few months of storage (Johnson, 1996). I have, however, not found any scientific support for this suggestion.

In Figure 2, showing the germination percentage corresponding to each moisture content in the experiment, it seems that seeds at moisture content 33.4% germinate better than the other treatments. The difference is, however, not significant (p-value 0.2532 compared with fresh seeds), but interestingly short-term and rapid drying of recalcitrant seeds have been reported to increase germination rate and/or germination percentage (Pammenter *et al*., 1998, Tompsett and Pritchard, 1998). According to my results, this effect was not seen in yeheb seeds.

My assumption about the intermediate seed storage behaviour of yeheb is based on the proposal by Hong and Ellis (1996), that if most or all seeds tolerate dehydration to 10-12.5% moisture content, but further desiccation reduces viability, the seeds are probably intermediate. There was actually a decrease in germination percentage between 12.3 and 9.6% in my experiment, which suggests that yeheb seeds may not be dried below 10% MC, if not to risk the viability of the seeds. If yeheb seeds were orthodox, higher germination percentage and seed survival would probably have been seen for the moisture contents mentioned. If so, the seeds may tolerate storage for several months or years.

For yeheb seeds, there are probably no dormancy period following shedding. About 15% of the seeds germinated during the period, which elapsed between harvest and arrival in the laboratory, and we had no problems with the germination of fresh seeds. Seeds that germinated, did so within a month from collection date, and treatment with gibberellic acid and potassium nitrate did not stimulate any further germination, even if the tetrazolium test indicated that a few of the seeds were viable. However, there are no previous reports of tetrazolium tests on yeheb seeds and we did not do any tetrazolium test corresponding to the germination test of fresh seeds because of lack of seeds. There are still much to learn about the tetrazolium test procedure and evaluation on yeheb seeds, and the method must be studied further. Therefore, our conclusions about the viability of those three seeds that were enough stained for being considered as possibly viable according to the tetrazolium test, are quite uncertain.

Evaluation of methods used

In this study, silica gel was used to achieve dehydration of the seeds. The drying rate resulting from this method is "intermediate" compared to other methods, such as the equilibrium dehydration method (slow) or drying in forced-air circulation (rapid). It has been shown that slow drying rate affects viability negative in desiccation sensitive seeds and tissues, and positive in desiccation tolerant species (Farrant *et al*., 1993, Pammenter *et al*., 1998, Pammenter and Berjak, 1999). Desiccation tolerance in this study was assessed on whole seeds, which probably reduce the effect of drying rate compared to if excised seed tissues have been used for determination of viability. However, there is probably an effect, so all results on the desiccation tolerance of yeheb seeds discussed and presented in this thesis are valid only for seeds desiccated under the same drying regime.

As the seeds were not sorted in replicates according to size before the desiccation treatment with silica gel, the differences in germination and viability within and between different replicates with different TMC may be caused by uneven dehydration of individual seeds. Small seeds may have been at lower moisture contents than larger seeds and therefore, did not germinate. It is also possible that dehydration inside the seeds were uneven, so that the tissues necessary for germination were actually at higher moisture contents than the "mean" moisture content for the seed seen as a whole. These two factors combined may result in higher, or lower, germination percentage and viability within and among different replicates. However, during the germination tests, no obvious differences in germination capacity according to seed size were recognisable.

When determining the moisture content after the dehydration, only five seeds per replicate were used. The moisture content of each replicate was therefore depending on the size of these seeds. This causes some uncertainty about the moisture content for the individual replicates. Especially for the four replicates of 5% TMC, the moisture contents between different replicates varied much, seen as a large standard error of the mean. For these replicates, the moisture content varied between 5.5 and 14.5%, and the smaller the seeds used for the MC determination, the lower the result.

Future studies

From this study the only apparent conclusion that can be drawn are whether the yeheb seeds, as suggested by Johnson (1996), are recalcitrant or not. Further studies on desiccation tolerance and storage behaviour of yeheb seeds are necessary before any certain conclusions about yeheb seed storage category can be drawn. In such studies, it is important to prevent fungal contamination of the seeds if testing viability by germination tests. Five minutes in 1% sodium hypochlorite solution was not sufficient to escape fungal attacks. All non-germinated seeds with >24% moisture contents were dead since they were soft and spongy when pressing a finger upon them after totally six weeks of germination tests, maybe due to fungal infection. If the seeds had been properly desinfected prior to desiccation treatment and germination tests, it is possible that more of

them had survived. However, it may be the desiccation treatment that made the seeds more susceptible to fungal infection rather than the moisture content in the seeds. The absolute number of firm seeds with 9.6 and 12.3% moisture content was higher (17 and 11 seeds, respectively) compared to seeds with >24% moisture contents (no seeds) after the six weeks of germination tests. This may be a result of lower seed moisture content. Another explanation is that fewer of the seeds with 9.6 and 12.3% moisture contents had germinated at the end of the germination tests. The probability of "seed firmness" after six weeks of germination tests may be equal among the treatments, and therefore, non-dependent on seed moisture content. However, Hong and Ellis (1996) recommends storage at lower moisture contents and/or lower temperatures if fungal attacks during storage can be expected, since it would probably provide a more unfavourable environment for fungal growth than storage at higher moisture contents and/or higher temperatures. It would also be interesting to study the relation between the period of successful storage and seed moisture content. Depending on the the answer of the question whether yeheb seeds are recalcitrant or not, further research on appropriate seed storage conditions for long time preservation and germination will be made.

Yeheb is still a "new" agronomically usable species to the researchers. Much remains to learn about the biology, ecology, usage and preservation of this important multipurpose plant. It is of the utmost importance that further efforts are being made to save yeheb from extinction, especially since Ethiopia in recent years often suffers from droughts, making the yeheb plants even more attractive and more exploited because of lack of food, feed and fuel. It is not only a question about biological research, but also about politics and co-operation with the people living in the yeheb region. I hope people will be reasonable enough to realise the capacity of yeheb and take the threat of extinction of the plant seriously, and though make the necessary exertions to propagate and preserve the plant to the future.

Acknowledgements

This master's thesis was carried out at the Department of Ecology and Crop Production Sciences at the Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden. The study is a part of a project on domestication of yeheb (Cordeauxia edulis Hemsl.), financed by Swedish International Development Cooperation Agency (Sida).

First of all, I would like to thank my supervisor, Dr. Lars Andersson, for all his support and encouragement during my experiment and writing, and Dr. Asha Yahya for collection of seeds, copies of articles about yeheb, help with germination assessments, discussions and descriptions of usage of the plant. I also wish to thank the state of Ethiopia for permitting the export of yeheb seed, providing the genetic material for my study, and the villagers in Bokh, Ethiopia, that renounced 1 ha of yeheb plants for scientific purpose. Thanks also to Anneli Lundquist for views on amendments on the thesis.

My gratitude also to the personnel in SLU libraries for help with information search, copies of scientific articles and books, to Göran Bergquist and Liv Åkerblom for support with the statistical analyses and to Jonathan Sohl for help with shifting of germination dishes in the incubator. My special thanks to Niklas Jakobsson for listening to my progress and complaints during the study, for discussions, support and encouragement, and for giving me a hug when I seemed to need one.

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