Storage conditions of yeheb (*Cordeauxia edulis* Hemsl.) seeds

Sara Johansson

Handledare: Dr Lars Andersson

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Institutionen för växtproduktionsekologi
Sveriges lantbruksuniversitet

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Abstract

Yeheb, *Cordeauxia edulis* Hemsl., is an endemic, semi-arid shrub which has become over utilised and is today in danger of extinction. Its natural regeneration is severely threatened since it is used as seasonal feed for animals like camels and goats, fire wood and food for humans. To learn more about the shrub's biology and manage the regeneration of the shrub by seed propagation a SIDA financed collaboration project between the Swedish University of Agricultural Science, Ultuna and the Alemaya University in Ethiopia was initiated in 2002.

Seeds can be divided into three categories, depending upon their length of life, responses to desiccation and preferred storage temperatures. Orthodox seeds are best stored in low temperature and with low moisture content, recalcitrant ones are sensitive to both desiccation and often low temperatures while intermediate seeds represent something in between the former two categories. The length of time for which a seed can survive is genetically programmed but there are several factors that determine whether the seed will survive for this period of time or if it will die at an earlier point.

Finding the optimal storage conditions for yeheb seeds is of great importance since the seeds age and deteriorate quite fast and the ultimate objective is to manage the survival of seeds between two rainy seasons. The aim of this study was to find the best storage conditions for the seeds of yeheb. This was done in experiments by variating temperature (6±1 respective 16±3°C) and moisture content (in equilibrium with H_2SO_4 in concentration 25, 30, 35, 40, 45 and 50 % respectively). The germinability was then evaluated by germination tests after 4 and 6 months respectively. The experimental results showed that it was possible to store seeds while maintaining satisfactory germinability. There were significant differences in germinability between different water contents, as between the both storage temperatures. No significant differences could be observed between 4 and 6 months of storage but a small but still significant reduction in germinability was detected in comparison with fresh seeds. The best results were obtained in 16°C and with a moisture content of 10.2 och 12.3 %, equal to H_2SO_4 concentration 45 and 40 %, where the germinability reached 93.8 %. The experimental results indicated that seeds from the yeheb shrub could be classified as intermediate.

Sammanfatting

Yeheb, *Cordeauxia edulis*, är en endemisk halvökenbuske som har blivit överutnyttjad och idag betraktas som utrotningshotad. Dess naturliga föryngring är starkt hotad då busken bland annat används som betesbuske av kameler och getter, till ved och fröna rostas till människoföda. Genom ett samarbete initierat 2002 mellan SLU i Sverige och Alemaya Universitetet i Ethiopien genomförs ett SIDA projekt för att lära sig mer om växten och lösa föryngringsfrågan.

Beroende av sin livslängd, nedtorkningstolerans och temperaturpreferenser vid lagring kan frön delas in i olika kategorier. Ortodoxa frön lagras bäst vid låg temperatur och låg vattenhalt, recalcitranta frön är mycket känsliga för vattenförlust och generellt även för låg temperatur emedan intermediära frön utgör ett mellanting. Livslängden hos frön generellt är genetiskt bestämd men det finns en mängd faktorer som påverkar huruvida ett frö ska överleva så länge eller om det kommer att dö i förtid.

Detta examensarbete går ut på att genom lagringsförsök med variation av temperatur (6±1 respektive 16±3°C) och vattenhalt (i jämvikt med H_2SO_4 i koncentration 25, 30, 35, 40, 45 respektive 50 %) finna de bästa lagringsbetingelserna för yehebfrön. Att finna optimala lagringsbetingelser för yehebfrön är av stor vikt då de är relativt kortlivade, målet är att säkerställa (i första hand) den kortsiktiga lagringsdugligheten mellan två regnperioder. Livsdugligheten bedöms därefter med groningsförsök vid två tidpunkter, efter 4 och 6 månader. Resultaten visade att det är fullt möjligt att lagerhålla yehebfrön från en regnperiod till en annan med bibehållande av tillfredställande groningsförmåga. Det förelåg signifikanta skillnader i grobarhet mellan olika vattenhalter, liksom mellan de bägge lagringstemperaturerna. Däremot fanns det inga signifikanta skillnader i grobarhet mellan 4 eller 6 månaders lagring men en liten men ändock viktig minskning i grobarhet noterades i förhållande till färska frön. Bäst resultat uppnåddes vid 16°C för vattenhalterna 10,2 och 12,3 %, motsvarande H_2SO_4 koncentration 45 och 40 %, där grobarheten i bägge fallen nådde 93,8 %. Försöksresultaten indikerade att yehebbuskens frön kan klassificeras som intermediära.

Background

The importance of gene preservation

It is important to conserve all species and habitats for following generations and to preserve the stability of ecosystems since we do not know what will happen in the future. In the book "A color atlas of plant propagation and conservation" (1999) Rae and Ingram point out the fact that today, over 34 000 species or 12.5 % of the world's flora (higher plants like ferns, conifers and flowering plants) face extinction. Only during the last century as many as 1000 species have become extinct and the rate at which habitats are destructed is increasing. There are many reasons for saving threatened species. A very narrow base of approximately 20 crop species makes up human food. Many of these are highly specialised for a few production goals, leading to poor or hardly any genetic diversity. This makes the plants, and therefore us, very vulnerable. Wild related species may hold the genes required for disease resistance, higher yields, drought resistance or other properties needed in the future. Plants are also important indicators of environmental change. The flow of energy and the cycling of matter in ecosystems are major determinants of the quality of the environment; especially oxygen and water content of the atmosphere are strongly related to the photosynthetic activity of green plants. The economic aspects of humans' dependency upon plants for food, medicines, building material, cosmetics and tourism as well as aesthetical and cultural reasons are enormous. In western medicine as much as 25% of the drugs are derived from plants, however many are almost unknown outside the region where they are gathered. It may be presumed that there are more to be found since plants contain a great diversity of compounds. Also in urban areas plants provide shade, trap dust, reduce noise and slow winds as well as having a soothing effect.
In the developing countries, little of real substance will be achieved until the affected people themselves demand change and the causes of poverty are addressed. This will only occur when attitudes alter as a result of education (Rae and Ingram, 1999).

Ex situ plant diversity (plants grown/cultivated outside their native habitat/distribution area) are mostly agricultural and horticultural species and cultivars held for commercial purposes in seed banks, *in vitro* culture (tissue parts of plants grown/multiplied under sterile conditions in laboratories) and some field gene banks. Botanical gardens hold the largest collection of non-crop wild species, ca 4 millions accessions representing 80 000 taxa, most of these are held as living plants in mixed collections. Seed preservation in gene banks offers a complementary insurance to other conservation techniques (Maunder and Culham, 1999). A seed bank should contain a representative sample of the natural populations but it can not replace the need of adequate areas of land for gene preservation within natural ecosystems (Kolotelo *et al*, 2001). Orthodox* seeds are generally ideal for long term storage but the inability to store recalcitrant* seeds (* see page 9 for explication) over time is a problem since a viable seed stock is desirable in order to preserve maximum genetic diversity (Bewley and Black, 1985).

Short term storage is equally important. Farmers and growers must be able to store their seed and grain from one season to the next, otherwise new populations can't be established by seed. Several methods of transportation and storage

facilities are at use. Storage in the humid tropics presents the most difficult situation (mostly fungi and insects), and in the hot dry tropics and subtropics insects are the principal case of deterioration (Desai *et al*, 1997).

Part of a SIDA project

Yeheb (*Cordeauxia edulis* Hemsl.) is a long-lived (estimated in excess of 200 years), endemic, semi-arid shrub, which can withstand extreme drought. It's a multipurpose plant used as dry season animal feed, firewood, building material and for dyeing of textiles for garments. Most importantly, the yehebs seeds are used as an energy rich, valuable food (especially in droughts) for the local population in the border areas of Somalia and Ethiopia (Booth and Wickens, 1988).

Threatened with extinction

Overexploitation of the shrub by long term heavy grazing pressure, harvesting of seeds, cutting and fire but also erosion, droughts and war in the region has lead to pore, or none, natural regeneration and the plant is therefore threatened with extinction (Kazmi, 1979; Booth and Wickens, 1988; FAO, 1988; Assefa *et al*, 1997). Several trials have been carried out to grow yeheb in nurseries, but this is difficult; while the aerial parts grow slowly its fast developing taproot is sensitive and the seedling will die if the taproot is broken (Kazmi, 1979; Booth and Wickens, 1988; Nerd *et al*, 1990). In the 1970´s actions to protect the shrub was taken by the National Range Agency at the Somalian Government. All together 75 hectares where fenced to protect shrubs from grazing and some other areas where declared as reserves. According to Kazmi (1979) the situation had been improved and seed production was increasing, the outcome of the project is however unknown.

Aim of the project

This study is part of a three-year project financed by the research division SAREC at the Swedish International Development Agency (Sida). It's a co-operation between the Swedish University of Agricultural Sciences, Department of Crop Production Ecology, and Alemaya University in Ethiopia. The project goal is to find methods to manage the regeneration of the shrub by seed propagation and thereby making in situ, gene conservation, establishment easier within its natural habitat. The objective of this study was to investigate how the survival of the seeds were affected by temperature and water content during storage, i.e. finding the most favourable conditions for yeheb seed storage.

The shrub

Distribution and Climate

Native distribution of yeheb is restricted to open bush savannah in arid, semi-desert regions (Kazmi 1979; FAO 1988) of Central Somalia and Ogaden, Ethiopia (figure 1) and the growth area is declining. It has been introduced and is now growing in trial plots in Kenya, Sudan, Yemen (Veitmer 1985; FAO 1988), India (Veitmer, 1985), Australia, Israel, Tanzania, and USA (FAO 1988).

Yeheb is found to grow at an altitude of 100-1000 m on sands locally called `haud´ (Booth and Wickens, 1988). These homogeneous deep red sands have underlying red sandstones of continental non-salty origin and a high rainwater infiltration (Drechsel and Wolfgang, 1988). Soils are poor, extremely low in nitrogen and usually slightly alkaline (pH 6.7-8.4). Plants are never found in alluvial flats with moist silt or near water since it's intolerant to water logging (Booth and Wickens, 1988).

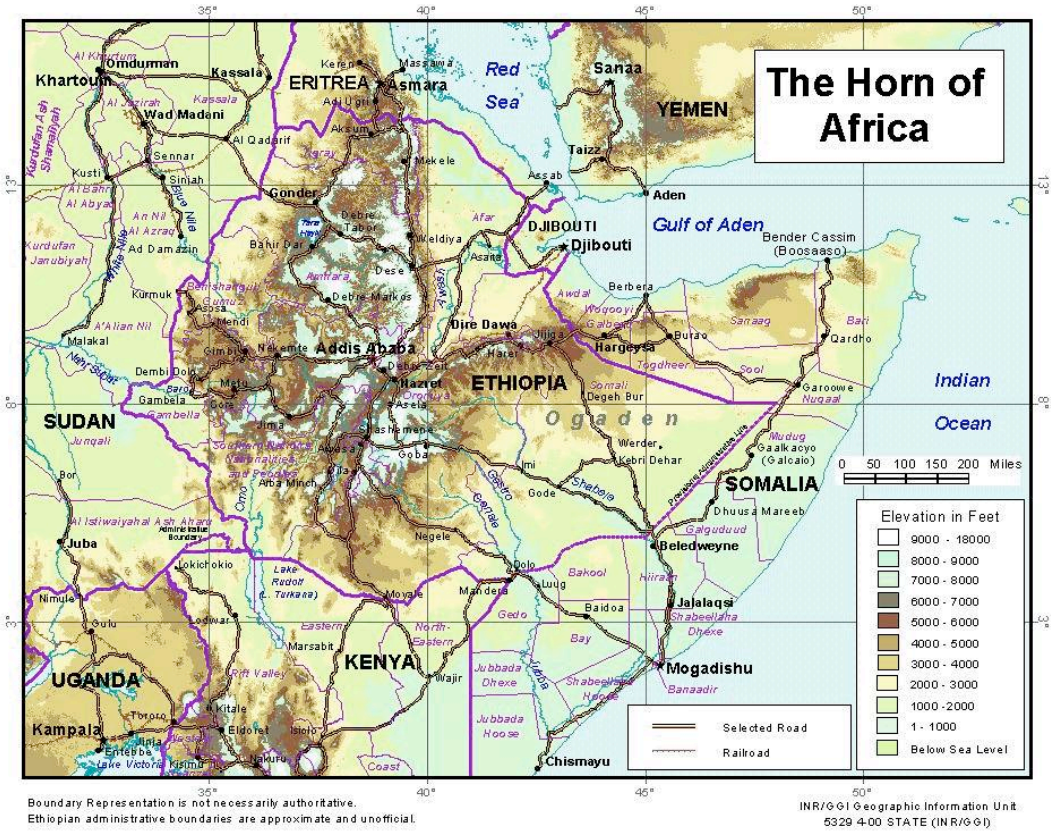

Figure 1. Map over the horn of Africa; Ethiopia and Somalia [Map from ReliefWeb www.reliefweb.int]

The natural distribution is frost free with mean annual temperatures of ca 28ºC (Drechsel and Wolfgang, 1988). Two rainy seasons, one more reliable (Yahya pers. comm. 2004) in March-May and another one in October–November, gives an annual rainfall of 85-400 mm. Plants can withstand extreme drought and water stress causes leaves to fold (inroll) and, in extreme conditions, to fall (Booth and Wickens, 1988).

Biology

Yeheb belongs to the family Leguminosae and the subfamily Caesalpinioideae. It is an evergreen, multi-stemmed shrub up to 4 m (Booth and Wickens, 1988), but usually its not higher than 1,6 m because of grazing (Kazmi, 1979). The fast growing tap root can reach depths of 3 m in search of moisture. The information differs whether yeheb has nodules or not. According to (Booth and Wickens, 1988) nodules have been reported on younger roots but Assefa *et al* (1997) did not find any nodulation on roots neither, in field excavations nor in rhizobial occulation trials in containers.

Two different forms of yeheb are recognised, usually in pure stands. The `Sulei´ form is smaller, usually less than 1,5 m with an open spreading habit and ovoid sweet tasting seeds of ca 1.8 g. The `Mogollo´ form is more common, taller and erect with less sweet seeds of 1.3 g (Booth and Wickens, 1988, FAO, 1988). Leaves are leathery, pinnate with red glands on lower surface and 1-3 up to 5-6 pairs of leaflets depending on form. Flowers are bisexual and yellow (FAO, 1988) with 4-6 cm long pods containing 1-2 up to 4 seeds. Seeds (mistakenly called nuts) are round to ovoid, 2-3.5 cm long with an average weight of 1.6g. Flowering starts when relative humidity increases, a few days before the rainy season. If abundant water/rain is available yeheb can flower twice but usually only once annually. The pollinating insect is not known but floral parts fall soon after pollination leaving only the fertilized ovary. Depending on rainfall fruits normally take 10-14 days to mature but it can remain undeveloped up to 4-5 months waiting for rains and then they can develop and ripen within 5-6 days. Yeheb seeds are self reseeding and germinate rapidly after dispersal, from a few days up to two weeks (Booth and Wickens, 1988). Seed viability is from several sources reported not to exceed a few months (Booth and Wickens, 1988; FAO, 1988). Information on germination rate differs significantly, seed condition are probably important. From only 30-50% (Assefa *et al* (1997) reported fungal problems) to over 70%-80% (Kazmi 1979; FAO, 1988) and to "seeds germinated well" (Nerd *et al,* 1990).
Much water is needed for establishment and initial tap root grow is given high priority while aerial growth is slow. A 60 cm tall plant might have roots reaching 2 m. Thus, once established, the shrub can survive up to 2 years without rainfall.

Shrubs are essentially free from insect pests but whitefly nymphs have been found feeding on stems. Seeds can be infested with different kinds of weevils and moth larvae. Non crop specific, storage pests such as dried fruit beetle, flat grain beetle, rust red flour beetle, tropical warehouse moth and Mediterranean flour moth have been found in seeds. There seem not to be any reports of fungal, bacterial, viral or physiological diseases (Booth and Wickens, 1988).

Propagation

Yeheb is self-reseeding and since the seeds are quite large and heavy they will germinate close to the mother plant. One hypothesis is that in nature, an earth squirrel may facilitate the establishment of new plants by burying seeds as a food reserve. Thus, giving forgotten seeds better germination conditions (Yahya, 2004). Nursery production from seeds is complicated and difficult since tap root is fast developing and the plant will die if it's broken (Kazmi, 1979; Booth and Wickens, 1988; FAO, 1988; Nerd *et al,* 1990).

It has been shown that exposed secondary rhizomes may regenerate vegetatively. Vegetative propagation by cuttings can be used, but no information about how successful the method is have been found; cut end is to be treated with fungicide and growth hormone and the rooting is induced under mist propagation (Booth and Wickens, 1988). Some pilot studies have been made regarding micro propagation but so far without greater success (Myers,1988; Yahya, 2004).

Use

Yeheb is a multi-purpose plant where most parts of the plant are used. The seeds are edible and eaten fresh, roasted, boiled or dried. Since yeheb seeds are easily damaged by fungi or insect attacks they are usually roasted to provide keeping qualities. Compared with other leguminous seeds, yeheb seeds are rich in fats (10-13 %) and sugars (12-25 %) but contain lower protein (11-16 %) and carbohydrate (31-41 %) levels. Also, protein quality is good since its rich in most essential amino acids (Booth and Wickens, 1988). Corresponding values given by FAO, (1988) are 11, 24, 13 and 37 % for fat, sugars, protein and carbohydrates, respectively. The seed yield of individual yeheb shrubs can be 5-8 kg per year, or none in drought years (Brink 2006). Somalis drink the sweet water remaining from boiled seeds and leaves are used for tea. In addition, seeds may be used as a coffee substitute. Wood is used as fuel wood by villagers and nomads (Booth and Wickens, 1988).

Plants may contribute up to half the biomass of the area and it is used by camels and goats as an important dry season browse. The estimated average forage production is 325-450 kg/ha (1.4-2 kg/plant) (Brink, 2006). Fodder value of the leaves is comparable to other tropical tree legumes but some mineral levels (P, Mg, Mn and partly Zn) would not satisfy the demands of animals if yeheb were the only source of fodder (Drechsel and Wolfgang, 1988). Herdsmen say that meat from animals feed on yeheb is particularly tasty and that teeth and bones of foragers become stained bright orange due to the cordeauxiaquinone present in leaves. Leaves have been used to dye cloths, calico and wool since the cordeauxiaquinone forms vividly coloured and insoluble combinations with many metals (Booth and Wickens, 1988).

Seed storage behaviour

Seeds can be classified into three different categories depending on their storage behaviour. According to Dickie and Pritchard (2002) seeds with orthodox storage behaviour make up the majority of seeds in the world, ca 90%, while ca 7% are recalcitrant and 2% are considered intermediate. It should be noted that this information is based on less than 2,5 % of all plant species, e.g. tropical moist forest species are under represented and that storage behaviour may vary within genera as for e.g. *Acer, Magnolia* and *Citrus* (Dickie and Pritchard, 2002). Orthodox seeds are best stored in a state of low moisture content and at low, generally subzero temperatures. Recalcitrant seeds species are usually found in tropical, sometimes in temperate, areas (Kolotelo *et al*, 2001). Their seeds are generally larger than orthodox ones, sensitive to low temperatures and must, to preserve their viability, be stored with high moisture content (Bewley and Black, 1985; Desai *et al,* 1997*;* Rae and Ingram, 1999; Dickie and Pritchard, 2002). Since

there are always exceptions a third “intermediate” storage category has been introduced. Here different seed species show a continuum of storage behaviour from very desiccation and/or low temperature sensitive to species tolerating quite low water potential and/or temperatures (Kermode and Finch-Savage, 2002). Seed size differs between the categories, however as in large scale comparisons the overlap is considerable. The mean 1000-seed weight for orthodox seeds are 329 g, followed by 900 g and 3958 g for intermediate and recalcitrant seeds respectively (Dickie and Pritchard, 2002).

Unlike many other plants yeheb shrubs flowers just before the onset of rains and the seeds mature when the plant moisture content is at its peak (Brink, 2006). Yeheb seeds have been reported not to retain viability for more than a few months, even if they are stored under ideal conditions and the recommendation has therefore been to sow them immediately. However, seeds coated in wood ash and stored in sacks are said to remain viable for at least one year (Kazmi, 1979; FAO, 42: 1998; Booth and Wickens, 1988). Based on embryo tissue survival after drying to MC in equilibrium with 15 % RH at 15ºC Flynn *et al* (2004) suggests orthodox storage behaviour. However, they confirm that viability of seeds is completely lost after a few months open storage at room temperature. Liew (2003) suggest intermediate storage behaviour.

Orthodox seeds

Temperature and moisture content of the seed are major factors in determining viability in storage. Seeds that can be stored in a state of low moisture content (MC of 1-8 %) and at low temperature are called orthodox (Dayan, 1997) and their viability under certain storage conditions conforms to some general rules;

-For each 1% decrease in seed moisture content the storage life of the seed is doubled.

-For each 5.6ºC decrease in seed storage temperature the storage life of the seed is doubled.

-The arithmetic sum of the storage temperature in degrees F and the percent relative humidity (RH) should not exceed 100, with no more than half the sum contributed by the temperature (Bewley and Black, 1985; Desai *et al,* 1997).

It is important to store the seeds in waterproof containers/dehumified atmosphere. If the storage RH is high seed gain moisture and if they are later brought out to a higher temperature they could deteriorate because of their high moisture content. For small batches of seeds, e.g. in *gene banks*, seeds could be kept immersed in liquid nitrogen (Bewley and Black, 1985).

Examples of orthodox seeds are *Brassica nigra* (Bewley and Black, 1985), *B. napus, Picea glauca, Allium cepa, Fragaria spp., Medicago sativa* (alfalfa)*, Canna, Lotus, Lupinus* (Desai *et al,* 1997), *Malus domestica* (Dickie and Pritchard, 2002), *Acer platanoides, Cucumis melo, Latuca sativa, Prunus cerasifera, Ricinus communis* and *Solanum tuberosum* (Flynn *et al,* 2004). In many cases, drying of seeds at a desiccation-tolerant stage of their development promotes germination upon subsequent rehydration. According to Kermode and Finch-Savage (2002) air-dried wheat grains germinate at an earlier stage of development and may also germinate at a faster rate than non-dried grains, though the rate, at which the seeds are dried at, is of vital importance for the survival and germinability of the seeds (Kermode, Finch-Savage, 2002).

Recalcitrant seeds

Seeds belonging to this category do not undergo maturation drying and are therefore shed at high water content. It is more accurate to express the degree of desiccation tolerance in terms of water potential as this reflects the amount of water available to the cytoplasm, but often it is expressed as moisture content (Kermode and Finch-Savage, 2002). At physiological maturity, the recalcitrant seeds have much higher moisture content (50-70%) than orthodox seeds (30-50%). The seeds are generally larger and continue to develop and progress toward germination (Desai *et al,* 1997), retaining active metabolism (Kermode and Finch-Savage, 2002). Since they are highly susceptible to desiccation injury they must maintain relatively high moisture content in order to remain viable. According to Dayan (1997) critical MC is generally 12-40 % but according to Dickie and Pritchard (2002) it is within the range 25-40 %. Also, low temperature storage can be inappropriate for recalcitrant plant species since many, especially tropical seed species are sensitive for chilling injury. Even when these requirements are met, their lifespan is often short and only occasionally exceeds a few months (Bewley and Black, 1985; Desai *et al,* 1997; Kermode and Finch-Savage, 2002). Some examples of genera with recalcitrant seeds are *Corylus, Castanea, Quercus, Aesculus, Salix* and *Juglans.* Many plantation crops and tropical species as coffee, kola nut, cacao, rubber (Bewley and Black 1985), *Cocoa, Hevea* and *Mango* are also recalcitrant (Mayer and Poljakoff-Mayber, 1989). Despite general adoptions for rapid germination, a few temperate recalcitrant species, e.g. *Aesculus hippocastanum and Acer pseudoplatanus*, are dormant at shedding (Kermode and Finch-Savage 2002). Even compared with equally sized orthodox seeds, recalcitrant seeds dry slower under the same conditions. Storage before drying results in a more desiccation sensitive seed and this may be caused by damage accumulated during storage or because storage has allowed the initiation of germination (Kermode, Finch-Savage, 2002). It is not known whether desiccation sensitivity of recalcitrant seeds is at least partially the result of an insufficient accumulation of protective proteins, or whether other factors (including a lack of protective sugars) are more important. Since desiccation tolerance is arguably a quantitative feature, the amount of protective proteins, or the rate at which the proteins accumulate, may determine the level of tolerance. Other features that may be part of the basis of desiccation sensitivity include an inability to repair desiccation-induced damage upon subsequent rehydration and inappropriate proportion or distribution of freezeable and non-freezable (bound) water within the seed (Kermode and Finch-Savage 2002). Kermode and Finch-Savage (2002) found a negative correlation between moisture content at harvest (premature and at shedding) and the moisture content at which 50% of the seeds from *Quercus robur* remain viable during drying. Seeds shed with the highest moisture content are the ones most sensitive to desiccation. This contrasts with orthodox species, where desiccation tolerance continues to increase after the acquisition of maximum seed dry weight, during maturation drying, which results in a metabolically inactive seed.

The physiological and biochemical bases for desiccation intolerance in recalcitrant seeds are not yet fully understood. So, at present, the optimal storage conditions for recalcitrant seeds can be determined only by trial and error (Bewley and Black 1985).

Intermediate seeds

The situation is complex with no clear cut line between recalcitrant and orthodox seed behaviour. The intermediate seed species is represented by all varieties in a gradual continuum from those extremely sensitive to dehydration/chilling and species that can tolerate substantial water loss/low temperature (Desai *et al,* 1997; Kermode and Finch-Savage, 2002). Generally they are more tolerant to desiccation than recalcitrant seeds but dried seeds loose viability faster at 0ºC and -20 ºC than around 15 ºC (Dickie and Pritchard, 2002; Flynn *et al,* 2004). Examples are citrus and coffee seeds (Desai *et al,* 1997).

Viability of seeds in storage

The longevity of seeds in storage is influenced by factors such as temperature, moisture and oxygen pressure. Generally, viability is retained best under conditions in which the metabolic activity of seeds is greatly reduced, i.e. lower temperatures and moisture content, whereas it is shortened by higher oxygen pressure for many species. Exceptions to these generalizations are the recalcitrant seeds which must retain relatively high moisture content in order to maintain maximum viability (Desai *et al,* 1997). Both desiccation sensitive and desiccation tolerant organisms are damaged when stored at intermediate water potentials. However, the time dependency of the damage varies considerably among species and tissues (Hong *et al,* 1998). Fluctuating storage conditions like altering high/low RH make seeds loose viability fast while altering high/low temperatures at intervals during cold storage does not necessarily have deleterious effects on viability (Bewley and Black, 1985). A study of the survival curves for seven species of seeds (wheat, rice, peas, barley, broad bean, tomato and onion) showed that under a given set of constant storage conditions a given sample of seeds has a mean viability period and that the viability periods of the individual seeds in a population are randomly distributed around the mean value. Under any constant storage conditions the frequency distribution of seeds deaths in time in normal, even in adverse storage environment (accelerated ageing) where seeds die rapidly (Bewley and Black, 1985; Desai *et al,* 1997).

Seed viability is not only a function of the conditions during storage. A variety of factors to which the parent plant is exposed during seed formation and ripening can also profoundly affect subsequent viability of seeds, after dispersal or harvest. Such factors include water supply, temperature, mineral nutrition and light. However, these environmental factors are less important than genetic control of seed viability (Mayer and Poljakoff-Mayber, 1989).

Water content

There are different strategies to cope with lower water potential. *Drought tolerant* plants resist water loss by having impermeable outer coverings and by reducing surface area-to-volume ratios they avoid desiccation. They cope with limited water availability while maintaining high internal water concentration. *Desiccation tolerance* plants cope with water loss. Owing to protection mechanisms (Hong *et al,* 1998) such as a reversible cessation of metabolism enables the organism to survive in spite of the loss of water (Alpert and Oliver, 2002). Depending on the cells degree of hydration five hydration levels can be defined (Figure 2). The cell

experience water stress when water potential is below -1 MPa but it continues to support growth until -1.5 MPa [level V]. During level IV (-1.8 to -4 MPa) stress related metabolism is affected but the cell continues to photosynthesize. *(Metabolism slows at water potential less than -2 MPa, protein synthesis slows at relatively high water potential, while respiration continues to much lower levels* (Hong *et al,* 1998)). Between -5 and -12 MPa [level III] respiration ceases and between -15 and - 190 MPa [level II] catabolic reactions stops. Around - 220 MPa and below [level I] the cell will be close to stasis (Hong *et al,* 1998; Walters *et al*, 2002). In non-dormant seeds moisture content above 30% will stimulate germination, whereas intermediate water contents of 30-18% gives ideal conditions for rapid deterioration caused by micro organisms. Seeds stored above 20-18% (see hydration level III) will respire and if kept in poor ventilation, the generated heat can kill the seeds. Water contents between 18-10% constitute the limit for fungi growth and destruction of seeds and below 9-8% there is little or no insect activity. Seeds with moisture content below 5-4% are immune from attack by insects and storage fungi but life span of the seeds is longer if they are stored at slightly higher moisture content (Bewley, Black, 1985). Many seeds can probably metabolize at a very low rate when maintained above 6% water content, and above 15% the metabolism of "dry" seeds might be substantial. According to Bewley and Black (1985) dry dormant wild oat grains can incorporate the radioactivity from radioactive ethanol into sugars, amino acids and proteins. In wheat grains with 18% water content decarboxylation and transamination reactions occurs (Bewley and Black, 1985).

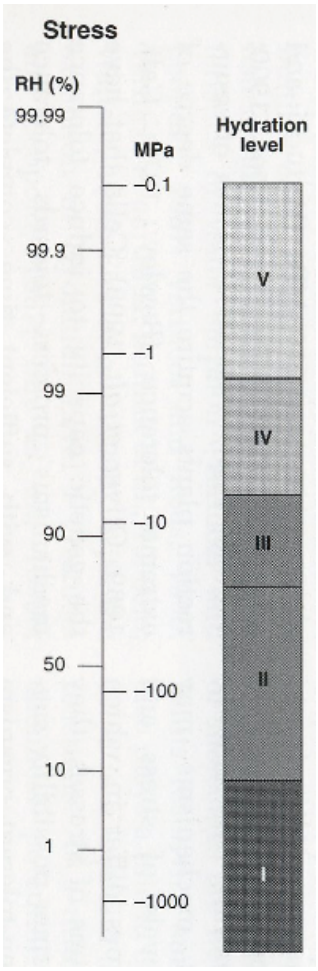

Figure 2. Scales of water stress; RH, MPa and hydration level (Walters *et al*, 2002).

In dry storage, the moisture content of seeds reaches equilibrium with the relative humidity (RH) inside the storage container. However, the equilibrium reached at a given RH varies with the species and depends on the chemical

composition of the seeds (Bewley and Black, 1985, Desai *et al,* 1997). Moisture content of different seeds may be different at the same RH, e.g. at RH 45% oil containing seeds (e.g. rape) have a moisture content between 4 and 6 % whereas starch containing ones (cereals and many legumes) have a moisture content of approximately 11% (Bewley and Black, 1985). Similar numbers are reported by Desai *et al* (1997). Many species have variable desiccation tolerance between tissues, e.g. seed, vegetative tissues and pollen, indicating that transcriptional, translational or post-translational control of existing genes is most probably involved in the expression of desiccation tolerance (Hong *et al,* 1998). Even within a species, seed tolerance to desiccation can vary according to provenience, but it is not known what proportion of this variation is genetic in origin or due to the environments in which seed development, storage or drying occurred (Kermode and Finch-Savage, 2002).

According to Kermode and Finch-Savage (2002) it is not known whether the ability to develop full desiccation tolerance has been lost in species with recalcitrant seeds, was never gained or is just not fully expressed. The cause of desiccation sensitivity is still far from understood. A number of processes have been suggested to be involved in the loss of viability of desiccation sensitive material and different processes may predominate in different water content ranges (Pammenter *et al,* 2000). At intermediate water contents, uncontrolled oxidative reactions can occur. Enhanced by inadequate protection by free radical scavengers and dependent upon unregulated metabolism they lead to desiccational damage like membrane damage (Pammenter *et al,* 2000; Kermode and Finch-Savage, 2002). At lower hydration levels the remaining water is non freezeable and is associated with macromolecular structures. Its removal can lead to conformational changes that may be irreversible and damaging (Pammenter *et al,* 2000).

In experiments with embryonic axes of *Quercus robur* Mycook *et al* (2000) found sub-cellular differences depending of the level of desiccation. A slight drying, from nominal 42-52% to 40%, exhibited an ultra structural profile similar to the control. Further drying to 35% gave the most obvious feature –increase in the extent of vacuolation in the cells of the root meristem. The endoplasmatic reticulum (ER) was dilated and the sheets of membrane had separated. The extent of the ER dilation varied among the cells and it is possible that these expanded sheets of ER ultimately formed the vacuoles. The cytoplasm remained dense but there where not much evidence of polysome formation which was found in the undried cells. Some aggregates of microfilaments where found. Material dried further, to 27% exhibited a wide range of damage (Mycook *et al*, 2000).

Upon drying induced compaction of molecules cell membrane must fold/vesiculate to accommodate the volume changes during cell contractions. This requires greater packing efficiency resulting in localized enrichments of similar type molecules in a process known as de-mixing. Molecules remix upon rehydration but the reactions that occurred in the desiccated state may have irreversible consequences. Thus, conservation of membrane surface area during contraction is critical for successful rehydration (Hong *et al,* 1998).

Temperature

Temperature and moisture content of the seed are major factors in determining viability in storage. Different species have varying requirements for seed storage temperature. Negative for the longevity of the seeds is generally high temperature

drying (Desai *et al,* 1997). Orthodox seeds are best stored at low moisture content and temperature between 0-5 ºC. Generally each 5.6ºC decrease in seed storage temperature doubles the storage life of the seed (Bewley and Black, 1985; Desai *et al,* 1997). For recalcitrant plant species no such general rule can be applied, but especially for tropical seed species low temperature storage can be unsuitable since many are sensitive to chilling (Bewley and Black, 1985; Desai *et al,* 1997; Kermode and Finch-Savage, 2002).

Most combinations of time, temperature and moisture content that lead to loss of viability of seeds during storage will result in some genetic damage in the survivors. In e.g. garden pea, barley and several other species this shows as chromosomal breakages appearing during the first mitotic divisions in the root tips. In forced ageing experiments with broad bean under high temperature (45 ºC and 18% MC) the mean viability period is less than one week. Lethal cytoplasmic lesions occur and there is a clear negative correlation between loss of viability and accumulation of chromosome damage in survivors. Hardly any of the aberrant cells persist beyond the first cell division but minor genetic damage like recessive gene mutations may persist, masked by their dominant allele. However, it is not understood why seeds accumulate chromosome aberrations. The difference between chilling tolerant and- intolerant seeds may be found in the protein composition or differences in more minor membrane components, e.g. sterols. In some species, seeds already exhibiting reduced vigour are more sensitive to chilling damage and the damages are more severe. This might be a consequence of ageing.

Storing seeds at higher temperatures can lead to attack from insect pests like weevils and flour beetles/borers. They are dependent on temperature and rarely active below 18-20ºC but mites have temperature tolerance close to 0ºC (Bewley and Black, 1985). Especially at high temperature micro organisms are likely to attack seeds and negatively affect their viability Desai *et al* 1997) but fluctuating temperature during intervals doesn't necessarily have deleterious effects (Bewley and Black, 1985).

Life span

The length of time for which seeds can remain viable is determined genetically. Environmental factors during growth and maturation, handling during harvest and storage conditions have a decisive effect on the lifespan of any given seed, i.e. whether the seed will remain viable for the full period determined by its genome or whether it will lose its viability at some earlier stage. (Mayer and Poljakoff-Mayber, 1989; Desai *et al,* 1997). Some seeds are genetically and chemically well equipped for longer storability than others under similar conditions e.g. *Canna spp, Lotus spp* and *Lupinus spp* while most species of agricultural crops are relatively short lived (Desai *et al,* 1997).

Seed ageing and subsequent deterioration of (orthodox) seeds includes a wide range of degenerative events that accumulate over time, causing loss of vigour and viability. These can be grouped as physiological and biochemical events. Physiological are decreased rates of germination and seedling growth, increased number of morphologically abnormal seedlings, decreased ability to emerge when sown under stressful conditions, degrading of functional structures, and depletion of food reserves, increased metabolite and ion leakage and greater susceptibility of seedlings to pathogens. At the biochemical level a decline in metabolic activity

upon germination, changes (in most cases decrease) in enzymatic activities, accumulation of toxic compounds, inability of ribosomes to dissociate, starvation of meristematic cells, genetic degradation (random somatic mutations) and a decline in protein and nucleic acid biosynthesis are seen (Bewley and Black, 1985; Desai *et al,* 1997; Bernal-Lugo *et al,* 2000). Changes in enzymatic and non-enzymatic oxidations include free-radical oxidations, enzymatic dehydrogenation, aldehyd oxidation of proteins, protein glycation (Maillard) reaction and changes in lipids induced by peroxidative processes. Lipoxygenases apparently can be active in seeds with very low water content. It is therefore reasonable to hypothesize that the cell defence mechanisms against oxidative damage may be also involved in maintaining seed vigour and viability (Mayer and Poljakoff-Mayber, 1989; Bernal-Lugo *et al,* 2000). In experiments with maize Bernal-Lugo *et al* (2000) found that ageing decreased the activity of the antioxidant enzymes SOD and catalase in dry seeds of two maize cultivars. Ageing impairs the induction of the antioxidant defence enzymatic system that is necessary for protecting the germinating embryo from oxidative stress injury. The level of this impairment is related to seed storage performance. The stability of the enzymatic antioxidant system varies between maize cultivars and may be associated with storage performance and ageing stability of the seed. During seed ageing, deteriorative reactions may occur in part during storage, and in part during the early stages of germination. The mechanisms involved in maintaining seed vigour and viability during dry storage and during early stages of germination, may include the efficiency of the enzymatic system for hydroperoxide detoxification (Bernal-Lugo *et al* 2000). Lesions in DNA and loss of membrane integrity have been reported. (Mayer and Poljakoff-Mayber, 1989; Bernal-Lugo *et al,* 2000) According to Mayer and Poljakoff-Mayber (1989) evidence for the ability of species to repair lesions in their DNA exists only for monocotyledonous species. The appearance of lesions in RNA, during ageing, has also been reported. Desai *et al* (1997) reported deterioration of orthodox seeds during storage. In these seeds a decrease in the activity of PEP carboxylase and, RuBP carboxylase and an increase protease activity was observed during storage. The increase of the protease activity was thought to be responsible for the decrease in activities of other enzymes. Leaching of water soluble sugars and leucine ^{14}C increased with seed deterioration. The enhanced leaching was attributed to membrane deterioration during seed storage. Before seed deterioration glucose was exclusively oxidized by the EMP pathway, whereupon a part of the glucose was oxidized by the pentose phosphate pathway. These changes were found to precede the loss of germinability. Freeze drying of certain seeds improved their longevity in storage if their initial moisture content before treatment is less than 15% (Desai *et al,* 1997).

When seeds lose viability the total sum of processes which lead to germination no longer operates properly, but parts of the seed can still be viable but unable to complete germination. Because of this chemical or histochemical methods devised to test viability are only partially satisfactory. Such tests can only check for one definite reaction which may to some extent be correlated with the eventual ability of the seed to germinate (Mayer, Poljakoff-Mayber, 1989).

Fungal infections

Fungal infections can decrease viability of seeds by the production of enzymes (including cellulases, pectinases, amylases, lipases, proteases and nucleases) that

destroy specific compounds in seeds, and produce toxins (such as phytotoxins, mycotoxins and tentoxin) which can cause the inhibition of germination, breakdown of membranes and increased solute leakage from seeds. Seeds infected with fungi can show discoloration, produce heat and develop mustiness and caking. Water solutable exudates, as well as volatile compounds, from seeds can promote the growth of fungi. As seeds age in dry storage, the production of these compounds increases and their chances of being destroyed by fungi increase (Bewley and Black, 1985; Desai *et al,* 1997; Baskin and Baskin, 1998). Impermeable seed coats (physical dormancy) are a strong deterrent to fungi, but seed coats that are permeable to water also help resist the invasion of fungi by serving as a mechanical barrier to the growth of hyphae and reduce the diffusion of compounds such as sugars, amino acids, ions and proteins that promote the growth of fungi in the vicinity of the seed and produce chemicals that inhibit the growth of fungi. Compounds associated with decreased germination or growth of fungi includes flavonoids and phenolic compounds. Tannins may also play a role in preventing infection of seed by fungi (Baskin and Baskin 1998).

Fungi can be divided into field- and storage fungi. Field fungi invade seeds during their development on plants, they need high moisture content for grow (33% for cereals) and hence are infective only under conditions where maturity drying fails. Examples are *Alternaria spp, Claudosporium spp, Fusarium spp* and *Helminthosporium spp.* Storage fungi are usually of genera *Aspergillus* or *Penicillium* and do only infest seeds during storage. They are not present before, even if seeds are left on plants standing in the field after harvesting. Each species has a sharply defined minimum of seed moisture content below which it will not grow (Bewley and Black, 1985; Desai *et al,* 1997; Baskin and Baskin 1998). The activities of seed storage fungi are ultimately more influenced by the RH of the interseed atmosphere than by the moisture content of the seeds themselves because different seeds have different chemical composition (oil or starch containing ones) (Bewley and Black 1985). During the storage experiments with yeheb several, visually different, fungi where observed. These where handed over to assoc. prof. Sadhna Alström at dept. Forest mycology and pathology, SLU in Ultuna for examination. PhD student Bertukan Mekonnen has during her work "Yeheb associated micro-organisms and their effects on its growth and pathogens" found several species of fungi, of these three species of *Aspergillus; A. niger, A. biciliate and A. versicolor* has been isolated (Alström, pers. com.2006).

At seed moisture contents that are in equilibrium with an ambient RH below 68% fungi will not grow, hence they are not responsible for deterioration that occurs at moisture contents below ~13% in starchy seeds and below 7-8% in oily seeds (Bewley and Black, 1985; Desai *et al,* 1997). Bacteria do also play an important role in deterioration of seeds during storage. But, since free water is required; most seeds would be induced to germinate and if fungi is present, it would tend to suppress the growth of bacteria (Desai *et al* 1997).

Tropical species/ recalcitrant seeds generally harbour very high levels of fungal propagules (Pammenter *et al,* 2002). Assefa *et al* (1997) used commercial fungicides to reduce the fungal infection on germinating yeheb seeds but they were unable to efficiently reduce it.

Other factors affecting longevity of seeds during storage

Other factors that affect longevity of seeds during storage are environmental factors during growth and maturation, handling during harvest, genetic differences within species and cultivars as well as micro-organisms and insects.

Environmental variation during seed development usually has little effect on the viability, unless the ripening process is interrupted by premature harvesting. Viability (of cereal grains) is generally lower in years when ripening and harvesting conditions is pore. Mechanical damage inflicted during harvesting can severely reduce the viability of some seeds (e.g. certain large seeded legumes) but small and/or spherical seeds tend to suffer less damage. Injured or deeply bruised areas may serve as centres for infection and can result in accelerated deterioration (Bewley and Black, 1985; Desai *et al,* 1997). Under inadequate storage conditions bacteria can grow, but since this require free water it makes them unlikely to grow on dried, stored seeds. Insects does not require so much water to be active, above 8% moisture content and 18-20°C, weevils, floor beetles, borers and mites can be a serious problem, particularly in warm and humid climates (Bewley and Black, 1985; Desai *et al*, 1997). Yeheb seeds are attacked by weevils and moth larvae. It is clear that insects are a serious problem during storage of yeheb seeds. Methods like roasting or boiling of freshly picked seeds are frequently mentioned in connection with seed storage. (Kazmi, 1979; FAO, 42: 1988; Booth and Wickens, 1988; Brink, 2006). Different cultivars and varieties from different harvests of a particular species may show different viability characteristics under the same storage conditions. Differences between harvests are relatively small under good storage conditions but under adverse conditions, such as elevated temperatures or RH, they can be quite large. Oxygen pressure may also change owing to respiratory activity of the seeds and associated micro flora even under conditions of constant temperature and moisture. By using hermetic (airtight) storage the seed moisture content can be controlled after the seeds have been dried adequately. For storage at relative low temperature and moisture there is probably little benefit in using controlled atmosphere, i.e. reduced oxygen pressure (Bewley and Black, 1985).

Biology of germination

Some recalcitrant seeds initiate germination related metabolism shortly after shedding. There is no clear cut event between the end of seed development and the start of germination, during both phases, recalcitrant seeds appear to maintain metabolically active (Kermode and Finch-Savage, 2002). However, germination *sencu stricto* begins with water uptake/imbibition and ends with the start of elongation by the embryonic axis, usually the radicle. The piercing of the seed coat by part of the embryo is caused by cell division, cell elongation or both. Processes such as mobilization of the major storage reserves are post germination events (Bewley and Black, 1985).

Imbibition

Total amount of water taken up by imbibition is generally quite small and may not exceed 2-3 times the dry weight of the seed (Bewley and Black, 1985). Depending on the dominating type of energy reserves, different seed species take up different

amounts of water (Baskin and Baskin, 1998). Under optimal condition water uptake in non dormant, germinating seeds goes through three phases; imbibition, lag phase and radicle elongation. When dry seeds starts to take up water they leak solutes such as sugars, organic acids, ions, amino acid and proteins into the surrounding medium. These solutes might stimulate the growth of fungi and bacteria in the soil which will invade the seed with risk for deterioration. For subsequent seedling growth, which involves the establishment of root-shoot systems, a larger and more sustained supply of water is required (Bewley and Black, 1985).

Cellular events during germination and seedling growth

Respiration, enzyme- and organelle activity, RNA and protein synthesis are fundamental cellular activities involved in the completion of germination and the preparation for subsequent growth.
In the imbibed seed there are three respiratory pathways active; glycolysis, the pentose phosphate pathway and the citric acid cycle and during germination a readily available supply of substrate for respiration must be available. Mitochondria in dry and freshly imbibed seeds are functionally and structurally deficient, they are characteristically poorly differentiated and lack cristae. In imbibed seeds there are two distinct patterns of mitochondrial development; repair and activation of organelles already existing within the mature dry seeds and biogenesis of new mitochondria. During lag phase II of respiration many seeds experience conditions of temporary anaerobiosis. This period in seeds during germination can last from a few hours to several days. Upon penetration of the enclosing structures by the radicle, the levels of anaerobic products decline as they are metabolized under conditions of increasing aerobiosis. Protein synthesis is essential for germination to be completed and for the radicle to emerge. Its commencement after imbibition is largely independent of prior RNA synthesis, but before the completion of germination newly formed RNA´s is probably needed for protein synthesis. A substantial number of enzymes are synthesized *de novo* in the mobilization of stored reserves in any storage tissue. Not all regions of a storage organ necessarily exhibit the same pattern of RNA metabolism. Expansion of the radicle within the seed occurs initially by cell elongation and its subsequent emergence through the seed coat may or may not be accompanied by cell division. Hence, DNA synthesis and cell division are largely post-germination phenomena, concerned with axis growth and establishment of the seedling (Bewley and Black, 1985).

Germination tests

A germination test has to be long enough to allow seeds sufficient time for germination, but it should be terminated after approximately two weeks so that dormant seeds shouldn't be able to get their stratification (Baskin and Baskin, 1998). An increased lag period before germination or a decrease in germination rate may indicate damage that is repaired during the lag phase, however seeds may produce a radicle (=germinated) but can be too damaged to be able to establish a viable seedling (Pammenter *et al,* 2002). Sometimes seeds that are normal in size, have endosperm, are firm when pinched and are not infected with fungi do not have embryos (Baskin and Baskin, 1998).

Material and methods

Pods of yeheb were collected in an area close to Bokh, in the Ogaden region in eastern Ethiopia (figure 1) during April 2004. Pods were picked from the shrubs when they had reached full size, but before full maturity. Pods were then gently dried in the shadow to 30-35 % moisture content (wet weight basis, wwb), to reduce the risk of fungal growth, before being placed in sacks of jute cloth. During transport to Sweden the sacks were kept in a box of strong corrugated cardboard. It's important to know that the seeds had not been exposed to extreme temperatures. Therefore temperature and RH was registered during the transport, and varied between 18 and 25°C, and 70 and 95 %, respectively. The visible condition of the pods seemed to be good, few had mechanical injuries or fungal growth.

Initial treatment

Immediately upon arrival at SLU, Uppsala May 18, all pods were peeled. Fresh weight of 4x25 seeds was measured to estimate the thousand seed weight (tsw). The tsw were calculated to 2979 g. Eight sub-samples of 5 seeds were taken to measure moisture content (wet weight basis, wwb) and water acitivity (a_w). Moisture content (MC) of the seeds was calculated by weighing 8x4 seeds before and after drying at 105° for 24h. Moisture content of the fresh seeds varied between 29.2-40.9 % with an average of 34.3 %. The last seed of each replicate, i.e. 8x1 seeds, was cut in smaller parts before measuring a_w with a Rotronic instrument. This showed a a_w of 0.903 (range 0.868-0.927). To evaluate the germinability of fresh seeds, a test was carried out on 10 seeds in 8 replicates. Each plastic germination dish (Ø = 16 cm) was filled with 330 ml sand (Backarps, Sweden), which had been sterilized for 24 h at 120°C and moistened with 105 ml de-ionized water. Ten seeds were evenly distributed in the dish, which was subsequently placed in a transparent plastic bag. All dishes were placed in an incubator with 12/12 hours light and darkness respectively and a corresponding temperature regime of 25/15°C. Light coincided with the high temperature. Every third day dishes were taken out and germinated seeds were counted and removed. Seeds were considered to have germinated when 2 mm of the radicle had emerged. The dishes were rotated in the incubator to prevent differences in light conditions. The intensity of light (photon irradiance) inside the plastic bag in the incubator was 35-40 µmol m-2 s-1

Table 1. Concentration of sulphuric acid and resulting relative humidity (estimation based on earlier measurements) in glass containers used in storage experiment, and final moisture content and water activity of seeds stored for 6 months

H_2SO_4 (%)	Estimated RH (%)	Final MC (%)	Final a_w
25	87	28.6	0.867
30	78	19.1	0.774
35	68	14.9	0.680
40	57	12.3	0.603
45	43	10.4	0.516
50	30	9.3	0.447

Storage experiments were conducted using 2 L glass containers ("Förvar", IKEA) with plastic lids sealed with parafilm. Storage was tested at 6 relative humidities ranging from 30 to 87 % (table 1) and at two temperatures, 6±1 and 16±3 °C. After 4 and 6 months the treatments were evaluated by germination tests. For each treatment (temperature x RH x time) 8 glass containers (replicates) were used, with 15 seeds in each container. For these experiments H_2SO_4 were used to obtain the different RH values. Non saturated solutions have limited buffering capacity compared with saturated salt solutions. In non saturated solutions the salt concentration changes slightly due too the equilibrium between tissue, vapour phase and solution phase. One way of reducing the risk that RH would not stay constant was to use 150-200 g solution/g tissue. The equilibrium relative humidity of the saturated salt solution of H_2SO_4 is not affected by the temperature in the range 5-40°C. (Sun, 2002) However, according to Andersson (2006) earlier experiments have shown that 50 ml is enough to maintain the buffering capacity of the H_2SO_4 solution and that the equilibrium change is neglectable. Fifty ml H_2SO_4 at varying concentrations (table 1) was added to each container and a plastic shelf was firmly placed above the surface to hold a plastic net basket with the seeds. Sterilization of seeds was made last when all bowls were prepared. The seeds were sterilized in a 5 % solution of sodium hypochlorite for 5 minutes and then rinsed 5 minutes in de-ionized water. As the seeds varied largely in size they were sorted into two categories, small-medium and large. When filled, each glass container was sealed with a plastic lid and parafilm to prevent diffusion. Half of the containers were placed at ~6°C while the rest were placed at ~16°C, all in darkness.

Germination tests after four and six months

After four (September 20) and six (November 30) months the storage experiments were terminated, giving 96 bowls and 1440 seeds per date to test. Mc and a_w was determined on 4 and 1 seed(s), respectively as described above. The germinability of the remaining 10 seeds per container was tested, using the method described above. The large number of dishes made it necessary to initially staple them randomly in 3-4 layers in the incubator. Every third day the dishes were stapled in

a new random order. The germination test was terminated after 14 days (October 5 and December 14) when the last counting of germinated seed was made.

Some larvae where found when storage periods where ended and seeds taken out for analysis and germination tests. These larvae where handed over to Dr Bert Gustafsson, Naturhistoriska Riksmuséet, Stockholm for examination. The larvae were identified as belonging to either Pyraloidea or Tortricoidea.

Viability test of non-germinated seeds

To establish whether the seed was dead (most likely due to fungal infection) or not, a needle were used to feel the consistency/resistance of each seed. Ungerminated seeds were considered dead if they appeared to be soft and/or if droplets became visible at pressure or puncture with a needle. To test if ungerminated, firm seeds were still alive they were placed in a giberellic acid (GA) solution. This was made to stimulate them to germinate, if they of some reason where in dormancy. Seeds were first washed to remove fungi and sand, then rinsed in de-ionized water and placed in a GA solution of 1% for 24 hours. A new germination test was made under the same conditions as before. Seeds that had not germinated after one week were considered to be dead.

Statistical methods

The statistical analyses were made using the GENMODE program (SAS Institute Inc. 1999). The material was handled as a binomial distribution with a logit link function divided into two classes (H2 and TEMP) and 6 + 2 levels (H_2SO_4 conc.; 25, 30, 35, 40, 45, 50, and TEMP: high/low).

By mistake the H_2SO_4 concentration in glass containers marked date 1, H_2SO_4 35 %, was too low resulting in a MC of 22 %, as compared to 15% at date 2. Because of this data from Date 1 (4 months storage) was omitted from the statistical analyses.

Results

Effect of moisture content and temperature

The statistical analysis revealed a significant difference among moisture contents (figure 3, table 2). The storage RH condition is an important factor when controlling the survival of the seeds. There were clear differences in the germination ratio among temperatures after 6 months (Figure 3). These observations were confirmed by the statistical analysis (table 2) showing clear significant differences between high and low temperature storage after 6 months. The ANOVA analyse also reveals significant interactions between different moisture contents and storage temperature (Table 2 and Figure 3).

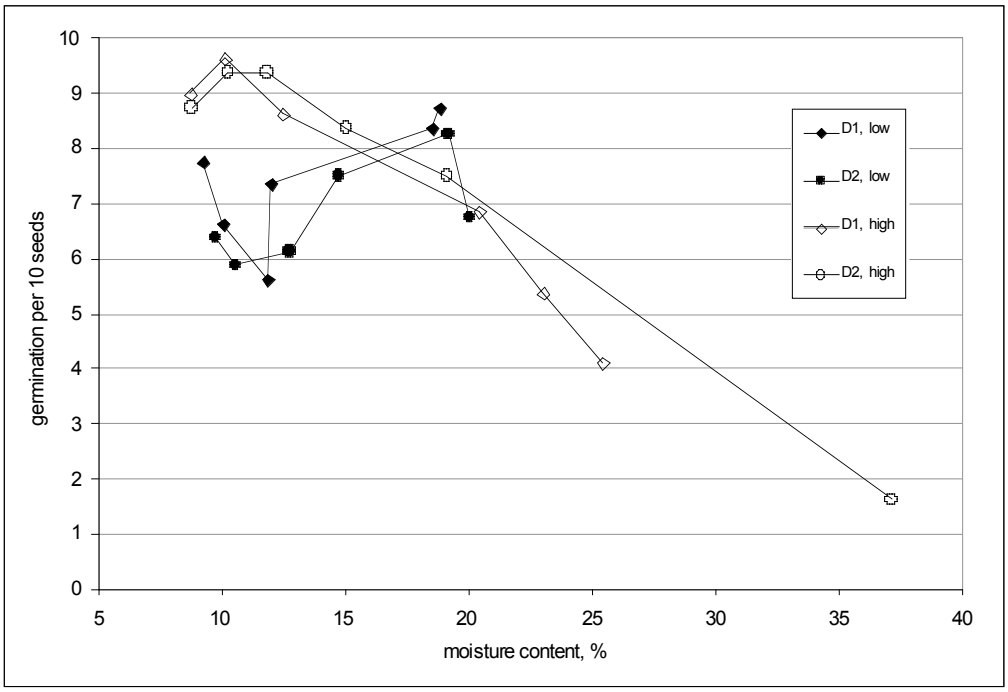

Figure 3. Germination ratio of yeheb seeds after storage over sulphuric acid. Seeds were stored during 4 (date 1) or 6 months (date 2), in 6 or 16ºC (low and high temperature). Each moisture content (8.7-37.1 %) corresponds to 8 replicates with normally 10 seeds per replicate.

Table 2. Maximum-likelihood ANOVA table for germination of yeheb seeds after storage over sulphuric acid during 6 months at six water contents and two temperatures

	d.f.	Chi-square	*p*
Temperature	1	13.6563	0.0002
Moisture content	5	73.6081	0.0001
Temp x Mc	5	183.8966	0.0001

The analysis between the best stored seeds and fresh yeheb seeds (table 3) revealed that there was a significant change in germinability. The germinability of the stored seeds had been somewhat reduced compared with that of the fresh seeds.

Table 3. Maximum-likelihood ANOVA table for viability. Comparison between the two best storage MC, H_2SO_4 concentration 40 and 45 % at high temperature (date 2), and the initial germinability of fresh yeheb seeds

source	d.f.	Chi-square	*p*
moisture content	2	6.6949	0.0352

Larvae

When storage periods where ended and seed brought out for tests several larvae belonging to either Pyraloidea or Tortricoidea where found. Most of them where found in the acid solution, more or less dissolved, but some where still in the net basket or even in the seed when these where cut into pieces. All together 55 larvae where found in 43 of 182 glass containers (Table 4). This gives (55 larvae/2880 seeds) 1.91 % insect infestations.

Table 4. Number of larvae per date (1 and 2) and temperature (low/high).

temperature	date 1	date 2	
low	12	3	15
high	15	25	40
	27	28	

Discussion

Finding the optimal storage conditions for a species such as yeheb is a diversified problem that needs to be examined in many aspects. The individual experiments in this SIDA project together will hopefully give some more understanding of how to handle yeheb seed desiccation and storage.

Storage conditions

According to Dickie and Pritchard (2002) different aspects like optimal storage temperature and desiccation tolerance, longevity of seeds in storage, seed size and "fleshiness" as well as ecological habitat of the mother plant must be taken in consideration when determining the storage category and optimal storage conditions for a species. Consistent with previous reports yeheb seeds loose viability after a few months (Kazmi, 1979; FAO 42: 1998; (Booth and Wickens, 1988; Flynn *et al,* 2004). Most indicators suggests recalcitrant or intermediate storage behaviour; the large seed size (Bewley and Black, 1985; Desai *et al,* 1997*;* Rae and Ingram, 1999; Dickie and Pritchard, 2002), lack of dormancy, seeds shed at relatively high water content (Desai *et al,* 1997; Kermode and Finch-Savage, 2002; Brink, 2006) and better survival at higher storage temperatures are factors, which all support this. The fact that yeheb grows in semi-arid areas could indicate orthodox storage behaviour, but according to Dickie and Pritchard (2002) the hypothetical relationship between habitat and storage response has not been confirmed.

Seed viability is not only a function of the conditions during storage. Factors affecting the parent plant during seed formation and ripening as well as handling during harvesting and transport can also affect subsequent viability of seeds. This is earlier shown in experiments with *Quercus* where seeds harvested different years from the same tree had different desiccation tolerance depending on the seeds moisture content when mature/harvested (Kermode and Finch-Savage, 2002).

Storage duration

According to literature the germination capacity decreases fast after harvest. However, these results showed that it is possible to store seeds for at least 6 months without loosing so much vigour. We wanted to try longer storage periods but did not dare because we had limited time. The risk of failure, that no seeds would survive a longer storage period, seemed too big for us to take the chance. There was actually a small, but significant, reduction in germinabilty between fresh and stored seeds. This makes it interesting to further study the survival capacity of the seeds. Thus the major aim, to manage to store viable seeds from one rainy season to the next, is possible.

Moisture content

Different RH conditions give varying conditions for micro organisms as well as varying seed response. The moisture content of the seeds plays a significant role for viability and germinability. The lowest acid concentration, 25 %, corresponding to the highest MC, 28.6 %, is markedly worse for seed survival. This is in line with preferred limits for micro organisms, fungi and seed metabolism (respiration). The best germinability was obtained from seeds stored between 10-12 % MC at high temperature after 6 months. After date 2 there is a decrease in germinability when the moisture content exceeded ca 12 % and below 10 % the germinability also decreases. According to Dayan (1997) orthodox seeds do well between mc 1-8 % and recalcitrant seeds shouldn't be dried below a moisture content of 12-40 %, but Dickie and Pritchard (2002) states limits within the range 25-40 %. Intermediate seeds can be dried down to ca 10-12.5 % before damages occur (Liew, 2003). Since the seeds in my experiment could withstand some drying, but drying below 10% seemed to reduce the germinability, my assumption based on desiccation tolerance is that yeheb seeds are intermediate.

Temperature

There are significant differences in germination due to storage temperature. These differences between high and low temperatures were clearly significant at the second date of testing (6 months). During low temperature storage (ca 6 ºC) there was generally a slight decrease in germination percentage from the highest moisture content (corresponding to 25 % H_2SO_4) to the lowest mc. Possible the seeds were weakened by an inappropriate storage temperature and thus had problems withstanding lower moisture contents. At high (ca 16 ºC) temperature the opposite seemed to be applicable; here germinability increased with decreasing moisture contents except for the lowest mc (8.8 %). The decrease in germinability for this moisture content could indicate the break point for the beginning of dehydration damage. Higher storage temperature seemed to suit the seeds better, thus they were in a better condition and therefore could withstand desiccation to a lower level. This interaction between temperature and moisture content was also statistically significant. Seeds clearly showed different sensitivity to storage temperature depending on their moisture content. Orthodox seeds are best stored at temperatures between 0-5 ºC. Intermediate seeds are better stored around 15 ºC and they loose their viability faster at lower temperatures. The responses of seeds in my experiment were consistent with intermediate storage behaviour since a too low storage temperature and too low moisture content of the seeds negatively affected their storability.

Experimental design

Seed size

During peeling and distribution among glass containers it was observed that the seed size varied considerably. The seeds were therefore divided into two size classes (small-medium and large) to make it more fair. However since the numbers

were uneven, 5 S-M and 3 L repetitions respectively, unfortunately no calculations on this could be conducted.

Viability test of ungerminated seeds

Seeds that had not germinated upon the third and last reading date and still felt firm were prepared for 1 % GA dip. However during washing, many seeds lost their seed coat and I found that almost everyone had a greyish-brown colouration of cotyledons and radicle so most of these seeds were considered dead. Only a few seeds actually did undergo the GA treatment and none of these germinated upon subsequent germination test. The majority of seeds had germinated within 5 days from the start of the test and no seeds germinated later than 13 days from the start. Therefore it appears to be unnecessary to carry out these tests and the seeds that do not germinate within 14 days can probably be regarded as dead.

Sterilization of seeds

The seeds used in this study appeared to be in a better visual condition compared with seeds collected and studied 2003 (Liew). Still there were some damages and losses most likely due to fungal infections. Almost every seed stored at 6ºC, both storage durations, were in good condition visually when brought out from storage. The opposite was general for H_2SO_4 concentration 25 %, and some from 30 % at high (16ºC) temperature storage. These seeds were epiphytically infested with fungi when brought out from storage. In H_2SO_4 35 %, which by mistake held a too low acid concentration and thus a too high RH, the majority where epiphytically infested with fungi. Clearly, sterilization methods need to be improved. Since seeds are markedly better at surviving harsh conditions than other plant tissues there are several possible methods for seed cleaning. Disinfection of a seed eradicates fungal spores within the seed coat or in the inner tissues whereas disinfestation is the destruction of surface organisms like fungi, bacteria and insects. Mechanical methods do only remove infectious material mixed with seeds and most pathogenic organisms from the seed surface. Physical methods like hot water and water soak treatments and ultraviolet, x-ray and infrared irradiation are only effective against pathogens present on or in the seed. The sensitivity to injury from soaking for longer periods differs considerably among crop varieties. Mostly used are chemical methods, liquid, slurry, powder or pelleting (Desai et al, 1997). In disinfestations and dormancy breaking experiments with *Acacia mearnsii* de Wild. Martin-Corder and Borges-Junior (1999) found that autoclaving seeds for 20 and 25 minutes were enough to disinfest seeds and gave mostly normal seedlings. Shorter autoclaving time and treatments with hot water followed by 10 % sodium hypochlorite (NaOCl, in 10 or 30 minutes) and 6 % benlate (benomyl) or 70 % alcohol for different time durations were not sufficient to disinfest the seeds and resulted in high numbers of abnormal seedlings. Ramakrishna *et al* (1991) compared different methods and treatments for their effectiveness in killing micro- organisms on or within barley seeds. *Aspergillus flavus* inoculated as spores was virtually eliminated by surface sterilization with NaOCl but mercuric chloride ($HgCl_2$) did not give satisfactory results. The barley grains were first immersed in 95 % ethanol for 40-50 s and then tested in NaOCl concentration 12.5, 25 and 50 % (v/v) for 5, 15 or 30 min and all treatments and combinations reduced the fungi to approximately a few percent disinfestations. In subsequent germination test the germinability was somewhat reduced compared with the initial 60-80 %

germinability in some treatments. Five minutes surface sterilization did not affect germinability while 15 min did reduce germinability to 90 % of the initial in 25 and 50 % NaOCl. Independent of concentration 30 min reduced germinability to 61-68 % of that in the control.

Soon after transfer to the growing chamber visual fungi developed, in some cases rather quickly. This was particularly obvious after the 6 months storage. Since moisture increases the succulence of the host and thus their susceptibility to certain pathogens this affects the extent and severity of disease. Moisture also affects the fungal spore production, longevity and germination of spores. Unfortunately the plastic bags and the condensation from altering night and day temperatures resulted in maintaining the high RH. The resulting film of water covering the tissues is a prerequisite for the germination of fungal propagules (Agrios, 1997). With time the extent of the individual infection increased in extension and severity and some started to sporulate. In some cases the same replicate could harbor 3-5 visually different fungi, of these three different *Aspergillus* species where isolated.

Insect pests

There seems to be more larvae in the higher temperature than in the lower, whether this is due to sensitivity to chilling is uncertain. Neither the storage duration nor the moisture content seems to affect presence of larvae. No eggs or entrance holes were to be found visually, so it's difficult to say how many of the seeds that actually were infected. However, the exit holes were easily detected.

Future studies

I hope that this study will bring some more understanding of how to store yeheb seeds with maintenance of satisfactory germinability. However, the storability of seeds is just one step towards reintroduction into its native habitat. To successfully manage the reintroduction it is important with extensive studies of the shrub *in situ*, finding the best introduction sites and how to protect these from grazing and seed collectors. The reintroduction, survival and growth of the population will only be a long term success if the local people who are dependent of the yeheb shrub are invited to participate and have possibilities to affect how the work should be carried out.

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Personal communication

Andersson, L. (personal communication 2006) Dept. of Crop Production Ecology, SLU, Uppsala, Sweden
Yahya, A. (personal communication autumn 2004) Dept. of Ecology and Plant Production Science, SLU, Uppsala, Sweden

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http://www.reliefweb.int/mapc/afr_ne/reg/afrhorn2000.html
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