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For quality of life

Seed quality in genetic resources conservation

A case study at the Centre for Genetic Resources, the Netherlands

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CGN Report 2008/11

Centre for Genetic Resources, the Netherlands (CGN)

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Summary

This report describes an analysis of the impact of workflow and storage conditions at the Centre for Genetic Resources the Netherlands (CGN) on the quality of seed samples in their collection, with emphasis on seed longevity and health. First general information is provided on aspects of seed production and seed quality, next an analysis is made of the situation at CGN and the various crops.

In general the CGN curators are well aware of most aspects of seed quality. The quality assurance system guarantees that only seed samples with good germination capacity are included in the collection. Emphasis on seed health is presently restricted to viral diseases. Examination of a small number of seed samples showed that other seed borne diseases are present in or on the seeds. *Alternaria* type fungal infections were observed on seeds of *Crucifereae* and *Fusarium* type fungi with cereal seed samples. Considerable improvements in this aspect can be made, starting with a short seed health course for the curators.

The physiology of the seed at harvest has a great influence on subsequent longevity of the seeds. Less mature seeds or seeds that have started part of their germination processes have reduced longevity. CGN curators aim at harvesting mature seeds, in which they succeed for most crops. For those crops or accessions where seeds are shattered at maturity and the seed production is in the field, the seeds are harvested immature. With some crops the seeds are allowed to dry slowly, finish maturation and increase in longevity. However, with several crops the immature seeds are presently force dried and for some crops even with heated air. This latter practice is expected to have a negative impact on longevity of the seeds and initial slow drying without heating is recommended.

Onset of germination showed to be a more serious problem with *Crucifereae* and cereal crops than expected by the curators. Even pre-harvest sprouting was observed in some samples. Since onset of germination processes reduces longevity, more attention is needed to address this problem. Production of seeds under dry climate conditions should be considered.

Between harvest and ultimate storage in the collection, the seeds are stored under different conditions varying between crops. Most agricultural crops are stored between harvest and cleaning for about half a year without any control of humidity. It is expected that those seeds loose a considerable part of their potential longevity during this inappropriate temporary storage. For these crops seed longevity can benefit from storage in a room with an electronic dehumidifier to reduce seed moisture content. A pre-cleaning of the seeds to reduce volume can be performed soon after harvest.

Seed quality control is presently limited to germination tests, performed by third parties, in which seedling quality is evaluated as normal, abnormal or dead. Attention for seed health is generally lacking and for dormancy rather limited. It will require not much efforts to note also seed borne infections during the germination tests. Retesting of samples showing poor germination by the third parties provides often different germination data. When germination is poor or seeds are infected it is useful that the curators perform a second test themselves. This will give the curators insight in the reasons for poor germination and improve their understanding of seed quality aspects. The latter knowledge can be used to improve seed production and handling. A short course on seed physiology aspects is advised.

Long term storage is presently performed under rather optimal conditions, in vacuum sealed laminated foil bags at -20 °C or 4 °C after drying the seeds to an equilibrium with 15% relative humidity of the air. Care should be taken that the storage bags remain moisture and oxygen proof on the long term. It is expected that further improvements can be made by drying at 10% relative humidity and reducing oxygen levels during storage. However research is needed to test this, especially with less mature seeds. To provide answers in a timeframe of a few years, such research requires new techniques to analyse in detail potential damage of the seeds instead of simple germination tests.

A summary of recommendations is provided at the end of the report. In an annex the observed situation for the different crops is described. A second annex lists the observation on the quality of a number of seed samples from the CGN collection. Although this list is not representative for the entire collection, it demonstrates the type of problems that can effect seed quality in the collection.

1. Introduction

In the frame of biodiversity preservation many efforts are spent in collecting, reproducing and storing seeds from crops and wild plant species. In order to be effective, these seeds should maintain viable during storage. The longer the viability of the seeds, the less efforts are needed to reproduce accessions and it reduces the speed of loosing genetic diversity. Viability of the seeds depends on the initial quality of the seeds and the storage conditions. For the end-users it is also important that the seeds are disease free.

In general desiccation-tolerant seeds store best at lowest moisture content and lowest temperature. At the Centre for Genetic Resources the Netherlands (CGN) long term seed storage takes place under dry conditions in vacuum sealed laminated foil bags at 4 °C or -20 °C and after equilibration of the seeds at 15% RH at 15 °C. There is still debate on potential risks of seed storage below an equilibrium of 10% RH. Next to temperature and humidity the partial oxygen pressure is also important, since most deterioration in seeds is through oxidation processes.

In addition to the storage conditions, seed viability is also influenced by the physiological condition of the seed. Experience with soybean seeds showed that maximum seed quality is often attained at the moment the seeds gained maximum dry weight, the end of the seed filling period. This moment was therefore called physiological maturity. However, in recent years it has become clear that for most seeds maximum seed longevity is not acquired at 'physiological maturity' but at the end of natural seed maturation drying (Hay & Probert, 1995; Jalink *et al.*, 1998). Thus, it is important to harvest seed as mature as possible.

When plants exhibit an extended period of flowering and harvest is done on a single moment, the harvest may contain many immature or less mature seeds. Moreover, when seeds are shattered at maturity, the harvest may be composed of mostly immature or less mature seeds. Fast drying of the crop fixes the physiological status of the seeds. Moderate drying can allow the less mature seeds to finalise their maturation as long as the moisture levels in the maturing seeds are high enough to allow metabolic activity.

It has also become clear that the onset of germination processes, prior to harvest, is accompanied by removal of protective mechanisms in the seeds, making the subsequently dried seeds more vulnerable to deterioration. Pre-harvest sprouting of the seeds will result in loss of desiccation tolerance, whereas onset of germination processes without radicle protrusion results in reduced longevity. The latter is influenced by the drying process of these 'pre-germinated' seeds.

1.1 Aim

The aim of the study was an analysis of the workflow and storage conditions at CGN that may impact the quality and longevity of the stored seeds. Although focused on the practices and conditions at CGN, general practices at gene banks worldwide will also be taken into account.

2. Seed quality and seed longevity in general

Seed quality characteristics are related to the user. Farmers require seeds that germinate uniform, under a broad set of environmental conditions and are free from seed borne diseases. Next to germination, the quality of the seedling is very important. For many commercial horticultural vegetable seeds a germination frequency of at least 95% and at least 90% normal seedlings are the standard. For genebanks other quality criteria are important. Maintenance of genetic diversity within an accession is the most important. The users, mostly breeders, need enough emerging seedlings to tests for the genetic trait they are searching for. Lower germination frequencies can be compensated by sowing a larger number of seeds. Although uniformity is important for phenotypic evaluation of genotypes, the need for uniform emergence is less stringent compared to the requirements by farmers. A very important seed quality characteristic for genebanks is the longevity of the seeds. Higher longevity needs fewer intermediate testing for decline in germinability and fewer cycles of multiplication. Especially multiplication is expensive and in general results in loss of genetic diversity.

Because of these different needs in seed quality, genebank seed production may differ from commercial seed production. For the genebanks total germination frequency may be lower and uniformity less, whereas longevity is of higher importance.

Production of high quality seeds is expensive and even more when it concerns a large number of small plots as with genebanks. With limited budgets available choices have to be made between spending more efforts and budget to increase seed quality or maximise the number of accessions that can be reproduced. It is therefore important to identify key factors in seed production and handling at genebanks that have a potential negative impact on seed longevity but can be solved with relative low costs or even without additional costs.

In general seed quality is determined by several factors: (1) seed health (pathogens in or on the seeds), e.g. *Fusarium* in cereals; (2) mechanical damage by e.g. insects, drying or threshing; (3) purity and (4) the physiological conditions of the seeds. The latter has a strong effect on seed longevity and is influenced by several factors: sink-source relation on the mother plant, applications of crop protecting chemicals, the climate during seed filling and maturation, seed maturity, pre-germination or pre-harvest sprouting, seed treatments, accumulated damage and stress tolerance.

A seed continuously accumulates damage, the amount of damage depends on the level of protection of the seeds against the biotic and abiotic stresses. During seed development and especially during maturation, seeds increase in tolerance against abiotic stresses. Once mature the seed has its maximum tolerance (Hay & Probert, 1995; Jalink *et al.,* 1998). The tolerance gradually decreases during its life time, e.g. through a decrease in anti-oxidant levels and accumulation of damage. A characteristic of living organisms is the ability to repair damage. Examples are the repair of DNA and cell membranes. Activation of the repair enzymes requires a minimum level of moisture and only starts upon imbibition of the seeds. A low level of damage accumulated during the dry storage is repaired relatively easy under optimal environmental conditions. Even with moderate damage a well growing seedling can be established, albeit with some delay in seedling emergence. However, when the accumulated damage has surpassed deleterious levels, it will effect the quality of the seedling and may even result in lack of emergence.

Protocols for handling during seed production, harvesting, cleaning and storage with gene banks should be aimed at producing healthy seeds with maximum longevity. Seed production should be aimed at harvesting seeds with optimal stress tolerance and seed handling should be aimed at reducing all types of stress that may result in the accumulation of damage.

3. Overview of seed production and storage procedures at the Centre for Genetic Resources the Netherlands

The process of seed production and storage can be divided in several steps. The development of the seeds, including maturation, harvesting, storage of the harvested crop or fruits, seed extraction, seed cleaning, temporary seed storage and long term seed storage. All these steps can influence the quality and especially the longevity of the seeds.
The CGN preserves germplasm of a variety of horticultural and agricultural crops. Each crop has its own type of seed development and requires dedicated methods of seed harvesting and cleaning. For part of the crops seed production is in greenhouses, while other seeds are produced in the open field.

3.1 Seed maturation

The crops under responsibility of CGN, vary in uniformity of flowering and seed development. With crops as cabbage the seeds are shed at maturity, while with pepper the mature seeds remain enclosed in the ripe humid fruit. With most CGN produced crops the seeds are harvested at the mature stage (Table 1), which is either done through harvesting of mature fruits, when the seeds are not shed (e.g. pepper, cucumber and faba beans), or by trapping shattered seeds. An example of the latter is lettuce where a perforated plastic bag is positioned around the inflorescence, in which the mature seeds are collected after shedding from the mother plant. Seed production at CGN for onion and leek is in the greenhouse, here seed loss is prevented through harvesting the inflorescence stalk with the developing seeds before seed maturation and shattering.

With clover, *Crucifereae*, grasses, potato and lupine the seeds are also harvested before maturation. Except for the horticultural *Crucifereae*, these crops are grown in the field and exhibit shedding of the seeds at maturity and it is very difficult to trap shattered seeds in the open field. Crops like those from the *Brassicaceae* flower over an extended period of time and consequently at a certain moment the plants bear seeds off all developmental stages. It is it is not feasible to pick individual mature fruits just before shattering, and the entire crop is harvested, containing seeds of all maturity classes except the mature seeds, that have already shattered.

Table 1. Diversity in harvesting either mature or less mature seeds for the various CGN crops.

Crops or species for which seeds are harvested mostly mature	Crops or species for which seeds are harvested immature or less mature
Cereals	Clover
Cucumber	*Crucifereae*
Egg plant	Grasses
Faba beans	Leek
Flax	Lupine
Lettuce	Onion
Pea	Potato
Pepper	
Spinach	
Tomato	

When harvesting of mature seeds is not feasible, the next best option is to collect seeds as mature as possible and to create conditions under which the seeds can finalise their maturation. This means a slow reduction of seed moisture content: enough drying to trigger the induction of protective mechanisms but slow enough to allow a minimum moisture level for metabolic activity to prepare the protection.

At CGN the harvested (immature) seeds from onion, leek and the grasses are slowly dried after harvest and the natural maturation process can at least partly be fulfilled. However, the seed harvest from *Crucifereae*, lupine and clover are dried fast with forced air and sometimes even at elevated temperature. The latter practice does not allow seed maturation after harvest.

It is advised to dry also these *Crucifereae*, lupine and clover crops more slowly, although care should be taken to avoid deterioration due to a too high humidity and high temperatures during drying. Especially when leaf material is enclosed in the bags, or when the material is collected under rainy conditions, the moisture level can be rather high. In those cases an initial faster drying might be better, but preferably not heated and not too fast, followed by slow drying. The bags should also not be placed in direct sun light to avoid overheating. It will require some skills, feeling for the system and experience to find an optimum drying condition, related to the initial moisture level of the harvested crops.

3.2 Onset of germination before harvest

When seeds sprout (root protrusion) before harvest they loose their desiccation tolerance. When germination processes start, but without root protrusion, the seeds still survive drying. Such seed can be compared to primed seeds and may initially germinate fast. This pre-germination will hardly be noticed in the first germination test. However primed seeds deteriorate faster during storage and the same is expected for seeds that have started their germination prior to harvest. Since the initial germination is good, such a deterioration will only be noticed after several years when a new germination test is performed.

Onset of germination prior to harvest can occur only when the mature seeds are in a moist environment and have no dormancy. With the crops produced under protection (glasshouses or tunnels) onset of germination may happen with the fruit vegetables and horticultural *Crucifereae*. Germination of rape seed or cabbage seeds within the siliques upon protected cultivation may occur under high humidity conditions (Groot, unpublished results). The CGN curator responsible for fruit vegetables is aware of those risks, especially for pepper and cucumber accessions with yellow coloured fruits and care is taken to avoid germination of the pepper seeds prior to harvest. This is mainly done through harvesting of less mature fruits with such accessions. No precautions are taken with *Crucifereae* seed production under protected cultivation.
With the field produced crops especially the *Crucifereae* and cereal crops are at risk. Examination under a binocular microscope of a few CGN seed lots from the 2006 production showed the presence of germinated seeds (pre-harvest sprouting) in some of the samples (Figure 1). The seeds with protruded radicle will be dead and will provide no seedling, but other seeds are expected to have started their germination processes and will have reduced longevity. The CGN staff was not aware of the risk and had never observed pre-harvest sprouting with seeds. One of the reasons may be that that samples are not routinely examined under a binocular microscope.
The outdoor grown *Crucifereae* and cereal crops exhibit high risks of pre-harvest sprouting or non-visible onset of germination when it rains in the period before harvest. Unfortunately this is frequently the situation in the Netherlands. For faba beans and flax no reports can be found in literature on pre harvest sprouting, Although it cannot be excluded that some accessions may have very low levels of dormancy. Nevertheless, the risks with these two crops can be considered low. With potato risks of germination before harvest are only present with a few known accessions lacking seed dormancy. Relative simple measures can be taken to reduce the risks with this crop.
Since most risks are expected with the cultivated cereals and *Crucifereae* crops, it is advised to have standard a close examination of the harvested seeds using a binocular microscope. Presence of germinated seeds may ring the alarm bell and for those samples the standard period before retesting germination capacity (survival during storage) should be shortened considerably. Since risk of pre-harvest sprouting has a strong genetic component, seed production of sensitive accessions should preferably be performed under dry weather conditions. This can

either be done under protection from rain (from end of seed development till harvest under a rain cover, or production under protected cultivation) or outside the Netherlands in a climate with dry summers.

Figure 1. *Pre-harvest sprouting observed in a CGN wheat (upper panel) and a yellow mustard (lower panel) seed lot.*

3.3 Seed health

When storing seeds for future use also seed health is an important aspect of seed quality. In this context the crops used for seed production which are vegetable fruits, lettuce, *B.oleracea*, radish, *Allium* and spinach are examined by experts from the Dutch Plant Protection Service for absence of viral diseases. Tomato seed production is in some cases monitored for absence of *Clavibacter michiganensis* bacterial disease, when the seeds are produced by some of the seed companies, but not at CGN.

The knowledge of CGN staff on seed borne diseases is rather poor and no special precautions are taken to avoid the transmission of fungal or other bacterial seed borne diseases. With seed samples produced in 2006 it was obvious that certain *Brassica* seed samples contained relative large frequencies of seeds infected with *Alternaria* fungi.

In wheat production in the Netherlands, contamination of seeds with *Fusarium* type fungi is rather common. For that reason commercially produced wheat seeds are almost always treated with fungicides. A CGN wheat seed sample from the 2006 production, that showed poor germination, was examined in a seed health test at Plant Research International (Figure 2). This sample showed indeed a large frequency of seeds contaminated with *Fusarium* type fungi. These fungi can have a large negative effect on seedling establishment. It is not known how these fungi survive during long term seed storage. It may very well be that they exhibit a lower longevity compared to wheat seeds and that the contamination is lost during storage. But is may also be that the fungi survive better and that wheat seeds weakened by long storage are more sensitive to infection with the fungi, or that infected seeds have reduced storability. The best is to prevent high levels of seed infection, by fungicide treatment during seed development. A total absence of *Fusarium* contamination is hardly possible in the relative wet climate in Netherlands. This can be an additional reason to propagate wheat and other cereals under more dry climate conditions.

It is highly recommended that a short dedicated course on seed pathology is organised for the CGN curators. Such a course should put emphasis on seed borne pathogens that are common to the CGN crops, on methods to monitor potential infection in the field during production or on the seed, on methods to prevent infection and on methods to cure the seeds if possible. The course can be provided by one of the seed pathologists of Plant Research International.

Figure 2. *Seed healthy test with seeds from a CGN wheat sample that had a low germination capacity. Only three of the 25 seeds produce a shoot. The other seeds are infected with Fusarium type fungi. P = primary infection without seed germination , Z = heavy infected seeds with signs of germination and † = dead seeds.*

3.4 Seed deterioration before harvest

With crops that do not shed (shatter) their seeds, the mature seeds can remain in the fruit (e.g. pod, silique or capsule) for some time till harvest and drying. Especially under humid conditions and/or high temperatures (irradiation by the sun), the seeds may start to deteriorate, even without onset of germination. It is as if the seeds are stored at high temperatures and at high moisture content. Also seeds that shatter and are collected in bags around the inflorescence (for example lettuce) can remain for several weeks in a relative warm and humid environment and are consequently prone to onset of deterioration.
It is advised to be aware of these risks during seed production and weigh these risks against advantages of extending seed production to collect more seeds.

3.5 Seed drying after harvest

As earlier mentioned in the paragraph on seed maturation, there is variation at CGN in the practice of seed drying after harvest. The different methods are listed in Table 2. With some crops the seeds dry largely on the mother plant, as for instance spinach and agricultural cereals. With other crops the seeds are harvested relatively wet, either mature (as with the fruit vegetables) or in different maturity stages (as with for instance leek and clover). The seeds are (further) dried either by air, by forced air or by heated (25 °C) forced air. Especially the agricultural crops are often dried by forced heated air.

Table 2. Different methods of drying the seeds after harvest, as employed by CGN for the different crops.

Initial drying method	Crop
Seeds extracted from the fruit after harvest and dried on air	Vegetable fruits, potato
On the mother plant protected from rain	Wild cereals, lettuce, spinach
On the mother plant exposed to potential rain	Cultivated cereals, faba beans, flax, pea
In the fruits (siliques, pods) attached to the cut mother plant, on the air	Grasses, leek, onion, horticultural *Crucifereae*
In the fruits (siliques, pods) attached to the cut mother plant, by forced heated air	Agricultural type *Crucifereae*, clover, lupine

It is obvious that fast drying of less mature or even immature seeds is not favourable for obtaining seeds with high longevity. Moreover, drying the non-mature seeds with heated air, as performed with some agricultural crops, may induce a further reduction of seed vigour, including longevity.
When visiting one of the two forced drying facilities, it was noted that the temperature was set at 30 °C, instead of 25 °C as written in the protocol, apparently due to lack of appropriate control. This is another risk of heated drying that can have substantial negative effects on seed quality.

3.6 Seed storage conditions before cleaning

Most often the seeds are not directly cleaned at harvest, which can be collecting individual fruits (e.g. vegetable crops and potato) or a large part of the mother plant (e.g. cereals and *Crucifereae*). The conditions and duration of storage from harvest till cleaning varies largely among the CGN crops. With potato and the fruit vegetable crops, the seeds are kept moist in the fruit in a cold place for a few days till several weeks and for potato subsequently also a few weeks at 20 °C. This is not so much a problem as long as the seeds are dormant.
The other crops are dried soon after harvest and stored some time before seed cleaning can take place. Depending on the crop and the availability of labour, it may take half a year before these seeds are cleaned. Although this

temporary storage is dry, the seed moisture contents vary considerable in relation to the storage conditions used. CGN has four types of temporary storage conditions before the seeds are put in the collection (Table 3).
The type of temporary storage is mainly related to the volume of the harvested material. Cleaned seeds have a small volume and can easily be stored in the drying room at 15 °C and 15% RH. The volume of the harvested material from leek, lettuce, onion, spinach, and the horticultural type *Crucifereae*, is small enough to be stored at 13 °C and 30% RH in the PRI seed storage facility. Harvested material from agricultural type *Crucifereae*, clover, faba beans, lupine, pea and wild cereals is stored in a large storage compartment with temperature control, but without control of RH. The harvest volume of cultivated cereals and flax is so bulky that this material is stored in large crates in a shed without temperature and humidity control.

Table 3. Available storage facilities for the harvested crop and seeds.

Storage facility	Crop
Large crates placed in a shed, protected from rain and frost, but without temperature and RH control	Cultivated cereals and flax
Large temperature controlled climate room set at 20 °C, but without control of RH	Agricultural type *Crucifereae*, clover, faba beans, lupine, pea and wild cereals
PRI seed storage room with temperature and RH control set at 13°C/30%RH	Horticultural type *Crucifereae*, leek, onion, lettuce and spinach,
CGN seed drying room	All seeds after cleaning

The higher the RH during intermediate storage, the higher seed moisture content and the faster the seeds deteriorate (see next paragraph). For maintaining longevity of the produced seeds, it is therefore important to store the seeds as short as possible under adverse conditions of relative high RH and occasionally higher temperatures. Half a year storage in the shed without humidity control is deleterious for the seeds.
It is useful to examine if the large volume crops cannot be (partially) cleaned faster. Even if only threshing is performed sooner after harvest, the volume is largely reduced and the threshed seeds can await further cleaning under temperature and humidity controlled conditions.
It is a pity that the RH is not controlled in the climate room used to store part of the harvested crop. The crop is brought in this room after (forced) air drying and initially the RH will be low. But by opening the doors and by human activities (respiration) in the room the RH will gradually increase and thereby seed moisture content. Exact control of humidity, using a humidity controlled climate room is rather expensive and not needed. It is only important to maintain a humidity of about 30% or less. In the presently used room the humidity can easily be reduced by placing an electronic de-humidifier in the room. The costs for such equipment is less than €500. This equipment is not meant for exact control of humidity, but to avoid a RH above 30%. Also a device for registration of RH should be placed in the room, e.g. an electronic datalogger.

Some staff from PRI is afraid that drying of CGN crops in their seed storage facility provides risks for their own seeds, especially by insects. Such risks are not unreal, since when visiting the climate room normally used for storage of the CGN crops, it was noticed that many small beetles were crawling in the bags with stored seeds. Fortunately, these seeds were not from the CGN collection.
When humidity in the CGN climate room is largely reduced electronically, this facility may also be used for temporary storage of the other CGN harvests for which presently the PRI seed storage facility is used. However, insect control remains important.

3.7 Seed deterioration during temporary storage

Storage conditions without control of the RH provide high risks of reducing seed longevity. In the non-controlled conditions under which the harvested flax and cereal crops are stored for several months awaiting cleaning, the temperatures are expected to fluctuate between 10 °C and 25 °C and the RH between 20 and 95%. As mentioned in the introduction seed longevity is influenced by seed moisture contents, temperature and partial oxygen pressure (Roberts, 1972). The moisture content of the seed is related to the relative humidity of the air (Figure 3). Different seed types show different so-called moisture sorption curves.

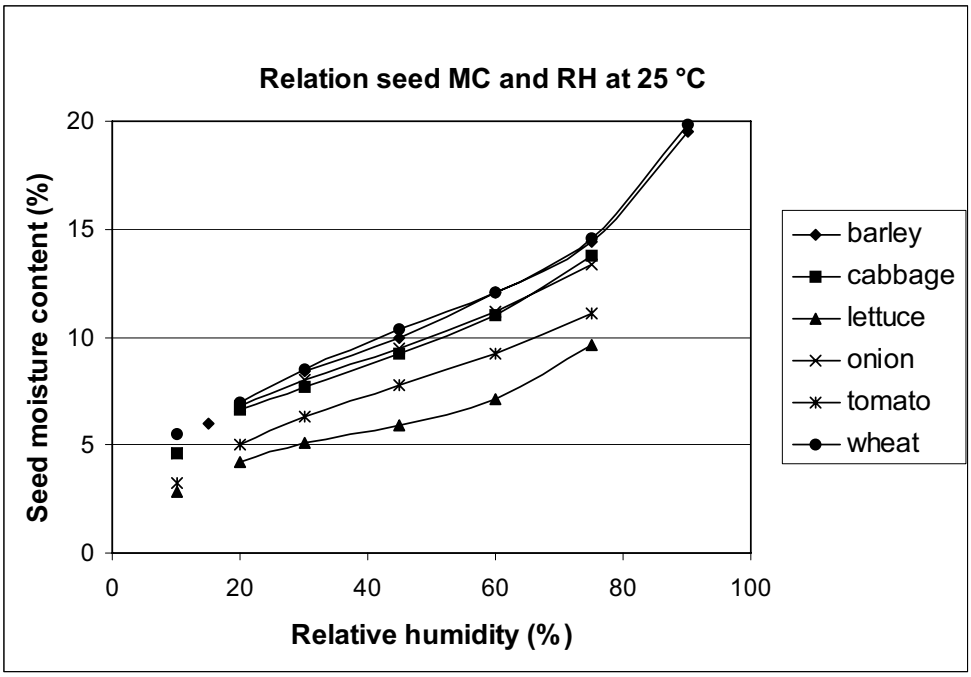

Figure 3. Equilibrium moisture contents of some crop seeds at 25 °C in relation to variation in the relative humidity of the air. Data from Rao et al., 2006, p. 39.

With dry storage of desiccation tolerant seeds, the relationship between seed longevity, temperature and moisture content can be quantified over wide ranges by the seed viability equation (Ellis & Roberts, 1980):

$$v = K_i - \frac{p}{10^{K_E - C_W \log m - C_H t - C_Q t^2}}$$

v = final viability (expressed as % or probits) after p days storage.
p = storage time (days)
m = % moisture content (fresh weight basis)
t = temperature (°C)
K_i = initial viability of the seed lot at p = 0 days (seed lot constant)
C_H and C_Q = species-specific temperature constants
K_E and C_W = species-specific moisture content constants.

It should be noted that the publications in which seed viability constants for species have been published, often a single seed lot from one variety has been used. Furthermore, because of time constraints accelerated ageing

conditions at relative high temperatures and moisture contents have been applied. For some species different publications are available, providing small differences in the viability constants. It is also known that longevity is influenced by the physiology of the seeds and also by genetic factors. As mentioned before, less mature seeds are more vulnerable to deterioration during storage.

The Royal Botanical Gardens, Kew provides a program on her web site http://www.kew.org/sid/viability/percent1.jsp, to predict storage time in relation to temperature and relative humidity during the storage. This can be simulated for 54 species for which published data on the viability constants are fed in the database.

This web based program has been used to predict the periods to decline from 95 till 50% viability upon storage at set relative humidity's and temperatures (Table 4).

The table clearly shows the accelerated deterioration effect when seeds are stored after harvest and before cleaning at non-optimal conditions. When the average relative humidity in the shed is 65%, the seeds deteriorate before cleaning more than 200 (*Phaseolus vulgaris* or *Brassica napus*) or 800 (*Allium cepa* and *Triticum aestivum*) times faster compared to storage at 5 °C in the collection. In other words, the theoretical decline in viability for the seeds stored at 20 °C and 65% RH for half a year is comparable to that during storage for about 100 or 400 years under the CGN collection conditions.

When the temporary storage is more optimised through shorter unfavourable storage periods and next under controlled low humidity conditions, the deterioration will go much slower. The largest advantage will be that it will take a much longer time before viability of the seed stored in the collection drops below the threshold urging a new multiplication.

The table raises two questions. First, according to the data from the Kew website the storability of *Allium cepa* seeds is comparable to that of *Triticum aestivum* seed and at low moisture levels to *Brassica napus* seeds. This is in contrast with the general experience at CGN and at the seed companies that *Allium cepa* seeds have a relative short storability compared to most other horticultural crops. This experience is confirmed by a publication of Walters *et al.* (2005) on germination data from seeds in the within the USDA National Plant Germplasm System collection mentioning half life values of 23 and 54 years for *Allium cepa* and *Triticum aestivum* seeds respectively. One reason for this discrepancy can be that the species specific parameters in the Roberts and Ellis seed viability equation are mostly based on artificial ageing tests at unnatural storage conditions (relative high temperature and moisture levels).

Table 4. Rate of decline in viability for four species in relation to the temperature and relative humidity at the storage conditions. The deterioration factor is calculated as the rate of decline relative to storage in the CGN collection (15 °C and 15% RH).

species	storage conditions	MC (%)	decline 95 -> 50% viability		
			days	years	deterioration factor
Triticum aestivum	75% RV / 20 °C	14.4	100	0.3	1074
	65% RV / 20 °C	12.8	121	0.3	888
	50% RV / 20 °C	10.6	602	1.6	178
	30% RV / 20 °C	7.9	3371	9.2	31.9
	30% RV / 13 °C	8.3	3362	9.2	140
	15% RV / 15 °C	5.7	40402	111	2.66
	15% RV / 5 °C	*5.7*	107401	294	1
	15% RV /-20 °C	*5.7*	472342	1294	0.23
Allium cepa	75% RV / 20 °C	13.0	95	0.3	1081
	65% RV / 20 °C	11.5	118	0.3	871
	50% RV / 20 °C	9.6	553	1.5	186
	30% RV / 20 °C	7.0	3467	9.5	29.6
	30% RV / 13 °C	7.5	3093	8.5	146
	15% RV / 15 °C	5.1	38645	106	2.66
	15% RV / 5 °C	*5.1*	102729	281	1
	15% RV /-20 °C	*5.1*	451798	1238	0.23
Brassica napus	75% RV / 20 °C	10.6	269	0.7	351
	65% RV / 20 °C	9.4	464	1.3	204
	50% RV / 20 °C	7.7	1149	3.1	82
	30% RV / 20 °C	5.7	4500	12.3	21.0
	30% RV / 13 °C	6	4750	13.0	87.5
	15% RV / 15 °C	4.1	21623	59	4.37
	15% RV / 5 °C	*4.1*	94545	259	1
	15% RV /-20 °C	*4.1*	415804	1139	0.23
Phaseolus vulgaris	75% RV / 20 °C	15.6	598	1.6	381
	65% RV / 20 °C	13.8	1071	2.9	212
	50% RV / 20 °C	11.6	2449	6.7	93
	30% RV / 20 °C	8.6	10181	27.9	22.4
	30% RV / 13 °C	9.1	10364	28.4	96.6
	15% RV / 15 °C	6.2	85605	235	2.66
	15% RV / 5 °C	*6.2*	227562	623	1
	15% RV /-20 °C	*6.2*	1000807	2742	0.23

A second point is that seed moisture content seems lower when stored at 20 °C compared 15 °C at the same RH of e.g. 30% or 15%. The present practice at CGN is to dry the seeds (before packaging and long term storage) at 15% RH and 15 °C. Based on the calculations using the Kew web site, it seems better to raise the temperature in the drying room to 20 °C, which will result in lower seed moisture contents. This idea is confirmed by Dr. Robin Pritchard (Royal Botanical Gardens, Kew, personal communication). It is useful to perform a test comparing these two situations. Such a test can be performed with an electronic controlled RH and temperature cabinet at PRI.

3.8 Seed cleaning

Procedures for seed cleaning are described in protocols according to the CGN quality management system. Seeds from fruit vegetables and potato are removed from the fruits by scraping or squeezing the fruits. The seeds are extracted using water (potato, eggplant and pepper) or hydrochloric acid (cucumber 2% and tomato 1%). Fruit tissue and empty seeds are removed by means of flushing. The washed seeds are dried on paper and after drying placed in a paper bag.

Dry seeds are cleaned by threshing and sieving. The latter removes also the smallest immature seeds. Empty seeds and other plant tissues is removed using a blower. In some occasions, especially with the agricultural *Crucifereae*, inclusion of seed sized clay particles is not avoided. Cleaned seeds are collected in a paper bag and stored at 15 °C and 15% RH till the germination data are available.

The seed cleaning techniques employed at CGN are rather basic compared to those applied by the seed companies, which use for instance gravity separators (to separate seeds based on their specific weight, separating empty seeds, insect damaged seeds, stones and other lighter or heavier materials from the seed lot), indent cylinders (separates the seeds based on their length and can be used for separating plant parts and weeds, or for calibrating seeds based on their length) and colour sorters (sorts seeds on the colour and contamination like discoloured, infected or damaged seeds, soil and weeds can be removed). A recent type of equipment for seed cleaning is the chlorophyll fluorescence sorter developed by Plant Research International, which separates mature from less mature and immature seeds based on residual levels of chlorophyll.

The main reason seed cleaning is restricted at CGN is the risk of modifying the genetic integrity by sorting, for instance if genetic variation in seed size or seed colour exists within an accession. Because of the lack of a more stringent seed cleaning, as applied in commercial seed production, the quality of the CGN produced seed lots is below the standard level of commercial seed production, although considerable variation exists between crops. In itself this is not too much of a problem as long as enough viable seeds are delivered to the clients. Whereas modern farmers require high and uniform germinating seeds, this is not the case with the users of CGN supplied germplasm. The CGN clients mainly require a minimal number of healthy seedlings to analyse the genetic variation present in the accession. Seed sample size for CGN clients is generally between 25 and 300 seeds. The seeds are counted using a seed counter, that does not discriminate in seeds size or shape.

3.9 Seed quality analysis

Seed quality is analysed by germination tests performed by NAK-AGRO for the agronomic crops and by Naktuinbouw for the vegetable crops and potato seeds. Most of the analyses are performed during winter or spring, the low season for these institutions. The germination test are largely according to the ISTA rules. Important deviations are that only two replicates of 50 or 100 seeds are used instead of four replicates of 100 seeds and that the final counting is extended if the number of germinating seeds still increases. The latter is to compensate for the relative lower seed quality compared to commercial seed productions for which the ISTA tests have been developed.
A first evaluation of the germination is made after a fixed number of days, when the total number of germinated seeds is counted. The second evaluation is made at the end of the germination test and the frequency of normal seedlings, abnormal seedlings and non-germinated seeds is determined. With the latter it is noted if the seeds are alive (hard) or dead (soft). With faba bean an incision is made in the non-germinated seeds to test for hardseededness. With potato it is noted if non-germinated seeds are swollen as indicator for dormancy.

The tests do not provide information about seed health and hardly provide indications about the reason for absence of germination, e.g. loss of viability. Moreover, the tests are performed with only two replicates of 50 or 100 seeds. As far as known, the replicates seem to be positioned next to each other on the germination table. Seed samples that show poor germination are submitted some time later for a new test, however, often different germination figures are obtained.

Seed health is not analysed. Although the germination tests employed will show most seed borne fungi when seeds are infected, this is not or only occasionally noted during the test. This is a pity since the additional labour involved in making notes on fungal infection and the type of infection is rather limited. It is advised to ask the testing stations to incorporate these qualifications in their report. This will inform CGN if more specific seed health tests are needed. The ISTA germination tests employed are intended for non-dormant seeds. But for CGN it is important to know if lack of germination is due to seed borne diseases, dormancy or vitality problems. Also because of the limited reproducibility of the tests for seed lots with poor germination, it should be considered that CGN performs all second test herself. This will provide the curators with information of the reason for poor germination, which can help them in improving seed quality for future seed production. As suggested before, increased knowledge about seed physiology and on seed borne diseases is a prerequisite and should be provided through dedicated training. Another point that should be kept in mind is that when a relative large fraction of seeds in the sample has initiated germination processes before drying, these seeds will behave more or less like primed seeds: rapid germination when stored for a short period. However, these seeds will deteriorate relative fast during storage. The present germination tests do not provide clues on storability of the seeds. It is worthwhile to develop additional tests for predicting shelf life of the seeds. The exact protocol and period of controlled ageing has to be determined for each crop. The date obtained from these experiments will provide answers on the longevity of the seeds and will reduce the frequency of sampling for seed survival during storage.

3.10 Seed storage in the collection

The cleaned seed samples are dried in the drying room at 15 °C and 15% RH, where a seed moisture content of 3-7% is reached after equilibration. The storage in this room is at least for several months to 1.5 year , till the germination data are available and there is time to do the packing of the seeds. Seed samples that reach the minimal germination requirements are included in the collection. The seed samples are packed in laminated aluminium foil bags under vacuum. The bags consists of 3 layers: the inner layer of 80 µm polyethylene, an intermediate layer of 12 µm aluminium foil and an outer layer of 12 µm polyester. Polyethylene is necessary to seal the bags, the aluminium is non-permeable to moisture and the polyester is used to give the bag its mechanical strength. Four different sizes of bags are used, depending on the crop and the size of the seed samples. For spiny spinach seeds cardboard layers are used to prevent puncturing of the seed bags. The seed collection is stored at either 4 °C or -20 °C.

Each accession has at least one sample for duplication, two for regeneration, four samples for germination tests and several user samples. There is usually one residual sample. Sometimes when the seeds are very large, two residual samples are made. Depending on the expected demand by users, the number of pre-packed user samples varies per crop from six to ten bags with 25-300 seeds. Pre-packing of user samples is considered to be more efficient and avoids repeated exposure of seeds to changes in ambient temperature during storage.

The equilibration at 15% RH is at the upper part of the recommendations by Bioversity International, that recommends genebanks to store desiccation tolerant seeds after equilibration drying at 10 – 15% RH, giving moisture contents between 3% and 7%, related to differences in the seed oil content (Rao *et al.*, 2006; Anonymous, 1994). The main reason why the upper limit (15%) is chosen, is because of the debate whether ultra-dry storage (after equilibration at 10% RH or lower) is indeed better, does not provide advantages or may even have deleterious effects (Walters & Engels, 1998; Ellis, 1998). Despite the fact that about 20 years have passed since the start of that debate, little progress has been made in solving the question whether drying at 10% RH is beneficial or provides risks.

Some publications have shown detrimental effects of drying seeds to ultra-dry levels using silica gel, followed by storage at different temperatures (e.g. Chai *et al.*, 1998). However, seeds from a large number of different Brassicaceae species survived well for almost 40 years ultra-dry storage in flame sealed glass vials with dehydrated silica gel, at temperatures between -5 °C and -10 °C (Pérez-García *et al.*, 2007). Also other investigations did not show negative effects of 10 year ultra dry storage at -20 °C (Hong *et al.*, 2005). It can be that in these studies which show no detrimental effects, either the critical moisture content was not reached, or other factors are involved. Ellis & Hong (2007) suggested that oxygen may play a role. Another reason for differences in results may be caused by

variation in the physiological status of the seeds. There are strong indications that less mature seeds are more sensitive to ultra drying (Groot, unpublished results). Also during the onset of germination the sensitivity of seeds to extreme drying increases (Hong & Ellis, 1992). It is highly recommended that research is performed to test the effect of dry storage at very, low moisture contents with or without oxygen, especially with less mature seeds. To provide answers in a timeframe of a few years, such research requires new techniques to analyse in detail potential damage of the seeds instead of simple germination tests.

There is serious doubt whether foil bag containers remain moisture proof on the long term. If seed moisture increases during storage, due to leaky containers, this can provide substantial risks for accelerated loss of longevity. Gómez-Campo (2002) reported alarming effects on this topic. He tested 40 types of containers, including laminated foil bags used by many gene banks, with dehydrated silica gel. Only sealed brass cans, 'Kilner' jars with rubber seals and flame sealed glass ampoules prevented moisture intake during the three year test period and the tested foil bags were not water proof on the long term. Hong *et al.* (2005) had also used laminated foil bags and observed that after 10 years of storage almost all seed samples stored at 20 °C had increased considerable in their moisture content from initially in equilibrium with 10% RH towards around 43% after ten years. The ultra-dry seed samples stored in the foil bags at -20 °C were still close to their initial moisture content. This indicates that temperature can have an effect on the long term permeability of the laminated foil bags.

CGN combines the use of laminated foil bags with vacuum. This has the advantage that leakage of the foil bags can be noticed. Till present the only leakage that has been observed was due to inadequate sealing of the bags. Also CGN storage is mostly at -20 °C, which may slow down potential deterioration of the foil bags. At -20 °C the RH is very low, so even when leakage could occur the seeds will not increase much in moisture content. For storage at 4 °C this is different. Nevertheless, it is important to keep awareness on this point, especially for long term conservation.

At very high moisture levels, oxygen is essential for seed survival, but at reduced moisture contents (air dried) an increase in oxygen levels reduces seed survival (Roberts, 1972; Roberts & Ellis, 1989). The damage that has been observed in seeds can be related to the DNA, RNA and proteins. Also the membranes from cells and cell organelles can be effected. This is why anti-oxidants are important in maintaining seed vitality. Research with Arabidopsis mutant seeds deficient in vitamin E production showed that this antioxidant is essential for seed longevity and for preventing lipid peroxidation during germination (Sattler *et al.*, 2004, The Plant Cell 16: 1419-1432).

It can therefore be expected that reducing oxygen levels during seed storage will aid in prolonging seed longevity. In the present practice at CGN oxygen is freely available till the seeds are stored in vacuum sealed foil bags. Thereafter oxygen levels are low and are expected to be reduced further during storage through oxidation of organic matter in the seeds. As suggested before, temporary storage of cleaned seeds could also be performed in vacuum sealed foil bags. Inclusion of an oxygen scavenger packet in the bags can aid in quickly reducing the remaining levels of oxygen in the bags.

It should be considered that laminated foil bags can vary in the barrier characteristics for both oxygen and water vapour (www.sorbentsystems.com/mylar.html). These quality aspects from laminated foil bags should to be kept in mind when packing seeds for long term storage.

4. General recommendations

Many of the practices performed at CGN are already in line with extending seed vitality. Based on the observations of the present workflow at CGN some general recommendations can be made. Some can be incorporated on the short term, others are more in a long term perspective.

Short term

- To obtain maximum longevity the seeds should be harvested at maturity, not earlier and not later. For crops where this is not feasible and part of the seeds is harvested immature, slow drying is recommended to allow the immature seeds finishing their maturation program and gain in stress tolerance. The main crops where this applies are the *Crucifereae*, lupine and clovers. Heated forced drying should be avoided. When much wet plant material is included in the harvest a partial non-heated drying should be performed. The duration of slow drying will vary with the crop and duration of seed development. During the slow drying heating of the material through sun light should also be prevented. Registration should be kept of the duration of the harvest in the drying cabinets and of the temperatures during the drying.
- Crops that are produced outside or with high RH under protection, have considerable risk of onset of germination processes prior to harvest, even pre-harvest sprouting may occur. The curator should be at least aware of this risk, for instance with *Crucifereae* and cereal crops. With sensitive crops of accessions, seed production elsewhere in dry climates should be considered.
- More emphasis should be placed on control of fungal seed borne diseases.
- To increase the knowledge of curators on seed physiology and seed borne diseases dedicated (one day) courses on these topics are advised.
- To avoid risks for the PRI seed collection, CGN seed harvests should not be dried or stored in the PRI seed collection room.
- After harvest and drying the seeds should be placed as soon as possible under conditions of low relative humidity. In the case of bulky harvests and lack of possibility to clean the seeds completely directly after harvest (flax and cereals) at least a partial cleaning should be performed soon, to reduce volume and allow temporary storage in a climate room at low relative humidity. Storage of seeds (harvest) in the shed for more than two weeks without humidity control should be avoided.
- In general, the period between harvest and storage under optimal conditions (as used for long term conservation) should be as short as possible, taking logistics into consideration. Temporary storage of the entire cleaned seed sample in large vacuum closed laminated foil bag at -20 °C should be considered.
- To acquire low relative humidity levels in the temporary CGN storage (climate) room an electronic dehumidifier should be placed in the room together with equipment to log the relative humidity over time. The relative humidity should be below 30%. After cleaning the seeds should be transferred to the CGN drying room (15% RH and 15 °C).
- In the storage/climate rooms measures should be taken to avoid insect damage.
- To increase knowledge of the CGN curators on the quality of the produced seeds, a binocular microscope should be placed near the CGN drying room.
- In the seed germination tests performed by he NAK-Agro or NAK Horticulture also fungal contamination of the seeds should be marked when it is observed.
- Seeds that show in the NAK tests poor germination behaviour or relative high contamination with seed borne diseases, should be retested by the CGN curators themselves to get an exact indicator of the reason for poor germination (dormancy, infection, viability loss). The additional advantage is that this increases the feeling from the curators for aspects of seed quality.
- Since CGN recommends their clients to apply an additional heat treatment before sowing the cucumber seeds, it should be questioned if CGN should keep performing a heat treatment prior to storage, especially because of its potential risk of reducing subsequent seed longevity.
- Drying the seeds to an equilibrium of 10% RH, instead of 15% RH, can be considered. Potential sensitivity of certain seeds, e.g. less mature seeds, should be tested first.

- A relative simple test should be performed to test if seed moisture content is lower when seeds are equilibrated at 20 °C instead of 15 °C before being packaged.
- Laminated foil bags can differ in permeability for oxygen and moisture. These characteristics for the bags used by CGN should be compared to alternatives and for instance the bags chosen for seed storage at the Seed vault Svalbard.
- Laminated foil bags are sensitive to deterioration on the long term and more at elevated temperatures. The bags in the CGN long term storage should be checked regularly (once per one or two years), especially those stored at 4 °C if vacuum is released.

Long term

- New tests that can predict longevity of seed lots should be developed. Such tests will aid in reducing general losses of accessions and in reducing the frequencies of multiplication.
- More research is needed to answer when seeds are more sensitive to extreme drying. CGN can stimulate to raise funds for such research.
- Oxygen is a main trigger for seed deterioration. It should be considered to store the cleaned dried seeds also temporally under low oxygen levels or hermetic before final hermetic storage in the collection. Application of oxygen scavengers can be considered, but research is needed.
- Investigate the possibilities of shortening the storage time after cleaning and before final hermetic storage in the collection.

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Appendix I Seed production and handling of the various CGN crops

Cereals

The main CGN cereal crops are *Triticum* (wheat), *Hordeum* (barley) and *Avena* (oat). Cereal seed production is divided in two ways. Because of seed shattering (brittleness) wild accessions are multiplied in a glasshouses. Cultivated accessions do not shed seeds and they are reproduced in the field, which is less expensive compared to protected cultivation.

Wild accessions

The seeds are produced in glasshouses and have no risk of rain during seed production. The flowering is rather uniform. A perforated plastic bag is positioned around the maturing ears, initially only closed at the bottom. Spikelets with mature seeds that are shed are collected in these bags. When the stalks turn yellow and dry, the bag is closed and the ears and part of the stalk enclosed in the bag are cut from the plant. At this moment most of the seeds are shed but depending on the accession also seeds can be left in the ears. The bags with harvested ears and seeds are dried directly after harvest by forced air (25 °C) in the drying cabinets. Next the bags with dried plant material is stored in the shed for a few months without control of air humidity. About six months later the ears are threshed and the seeds are cleaned using sieves, according to ISTA protocols.
The smallest seeds are removed. The cleaning is not performed till the single seed level, since most seeds remain in their spikelet, which contain one or more seeds. After cleaning the seeds are stored in paper bags at 15 °C and 15% RH, till the germination data are available and seeds can be packed and included in the collection. With several accessions dormancy is a problem in the germination assays.

Cultivated accessions

Seed production with the cultivated accessions is performed in the field. Foraging by birds is a problem, especially with barley prior to seed maturation. Another large problem is encountered upon wet weather in the weeks before harvest, resulting in lodging of the plants and a potential risk of seed deterioration. Together with bird predation this may result in an insufficient seed production.
Although it is known that some accessions are susceptible for pre-harvest sprouting, this is hardly observed. Nevertheless onset of germination processes prior to harvest cannot be excluded, especially in years with much rain prior to harvest. Most of the seeds are harvested with a combine harvester dedicated for harvesting small plots, this harvesting method includes threshing of the seeds. Plots that mature earlier or later are harvested by hand and threshed later. A few accessions that show an intermediate vernalisation requirement, express variation in the moment of flowering and consequently seed maturation. With those types individual ears are harvested when the stalk turns yellow and dry.
The harvested seeds, or in some cases the ears with seeds, are put in linen sacs and immediately after harvest dried by forced air at 25 °C. The dried crop is stored indoors, but without control of temperature and relative humidity. Seed cleaning is performed after about six months.
In some years the produced seeds show a general poor germination, indicating poor seed quality. When poor germination is observed, a new seed production for this accession is initiated.

Diseases

There are not much problems with diseases encountered, except for some mildew and rust. Seed borne diseases as *Fusarium* type fungi were not considered as a problem by the CGN curator.

Comments

- Seeds produced in the glasshouse have the possibility to mature without risk or getting wet. However, seeds produced in the field have a very large risk of deterioration prior to harvest, as result of lodging and risks of pre-harvest sprouting, upon wet weather conditions in the weeks prior to harvest.
- Storage after harvest and before cleaning in non-controlled conditions, will result in accelerated deterioration of the seeds and reduction of longevity.
- Commercial cereal production in the Netherlands suffers almost always from infection with Fusarium type fungi, especially with humid warm weather in the weeks before harvest. These fungi can decrease seed quality. It is useful to investigate if indeed CGN cereal seed production does not have fungal infection.

Practical observations:

- Seeds from three wheat multiplications from 2005, showing poor germination, have been studied under the binocular microscope. With two samples it was clear that most seeds had sprouted before harvest (Figure 3). With the other sample a few seeds had sprouted, many other seeds had a shriveled appearance. Some of the shriveled seeds were also pink (Figure 3). Seed health analysis showed severe infection with *Fusarium* type fungi.

Figure 4. Severe seed quality problems encountered in two CGN wheat samples with poor germination: pre-harvest sprouting (left) and contamination with Fusarium fungi (pink color). The latter was confirmed with a seed health test (see Figure 2).

Recommendations

- Seed storage before cleaning should be done at low relative humidity.
- Pre-harvest sprouting can easily be seen at harvest. Hand harvest of only non-affected ears is recommended.
- Take great care with forced drying using heated air.
- Study a representative number of cereal seed samples for potential Fusarium type fungal infections to analyse the size of this potential problem.
- Consider multiplication of cultivated cereal accessions in a country with a dry climate.

Crucifereae

For seed production the *Crucifereae* crops are divided in two groups, the horticultural types and the agricultural types. With the horticultural types seeds are produced in protected environments (glasshouses), which is more controlled, but far more expensive compared to open field seed production as performed with agricultural types.

Horticultural types

With most accessions flowering takes place is over a long period. Per accession about 80 plants are grown in one isolated compartment. When about half of the plants is flowering, bumble bees are introduced. Since the bumble bees survive only for a limited period, seed set stops when no living bees are left. When seeds mature, the siliques dry and will open and seeds may start to shatter. Therefore once a large number of dry siliques is observed, the inflorescence is cut from the plant and placed in a linnen sac. The harvested inflorescences contain siliques with seeds of different maturity. Directly after harvest (around August) the sacs are placed in a drying cabinet, where the seeds are dried by forced heated air at a temperature of about 25 °C for three till five days. The linen sacs with dried siliques and seeds are stored for some months in the PRI seed storage room (13 °C and 30% RH). In December or January the siliques are threshed and the seeds are cleaned using sieves, according to ISTA protocols. The smallest seeds are removed, but it is not known to what extend less mature seeds are removed. After cleaning the seeds are stored at 15 °C and 15% RH, till the germination data are available and seeds can be packed and put in the collection.

Agricultural types

Also here, most accessions flower over an extended period. Pollination is by local insects. When seed set is sufficient, the top of the inflorescence, with flowers and young developing seeds, is removed. Rain during seed production provides risks on quality decline, especially during and after seed maturation. There is hardly dormancy in the accessions. Pre-harvest sprouting or onset of germination without visible sprouting was never observed by the CGN curator. As with the horticultural types the seed crop is harvested when the first siliques start shattering. The harvested inflorescences with siliques are packed in jute sacs. This material contains pods from all maturity stages. In the case of the abundant presence of vegetative material as leaves, the harvest can be rather wet. The material is directly dried placing the sacs after harvest in a drying cabinet, where the seeds are dried at a temperature of about 25 °C, for three till five days. The jute sacs with dried siliques and seeds are stored in the shed for several months without control of temperature and relative humidity. In January or February the crop is threshed, the seeds are cleaned and stored 15 °C and 15% RH till the germination data are available and seeds can be packed and put in the collection.

Diseases

The curator has never observed seed borne diseases. The Plantenziektenkundige Dienst (Dutch Plant Protection Service) controls for virus infection during seed production. There is no control for other diseases. Recently some countries, for instance Canada, require a phytosanitary certificate stating the seeds are free from diseases as *Xanthomonas campestris pv. campestris*. At present a test for this pathogen requires seed samples of 10.000 seeds. This sample size is too high to perform with CGN multiplied seeds.

Practical observations:

- Close examination of several *Crucifereae* seed lots, using a binocular microscope, showed that six out of nine samples contained at least a few germinated seeds (Figure 1). There was no difference between production in the field or in the glasshouse.
- Cabbage seeds produced for the breeding department of PRI, under similar conditions as those for the CGN horticultural type *Crucifereae* sometimes suffer from pre-harvest sprouting. This is related to high relative humidity in the week prior to harvest. Since CGN seeds are produced under partly similar conditions, this can also be a reason for the observed pre-harvest sprouting with the CGN seeds.
- A seed health test of four seed lots revealed a frequent contamination of the seeds with *Alternaria* type fungi (Figure 5).
- In a previous study dedicated to analyse potential reasons for occasional poor germination of CGN *Brassica sp.* seed samples, also a frequent contamination with *Alternaria* type fungi had been observed.

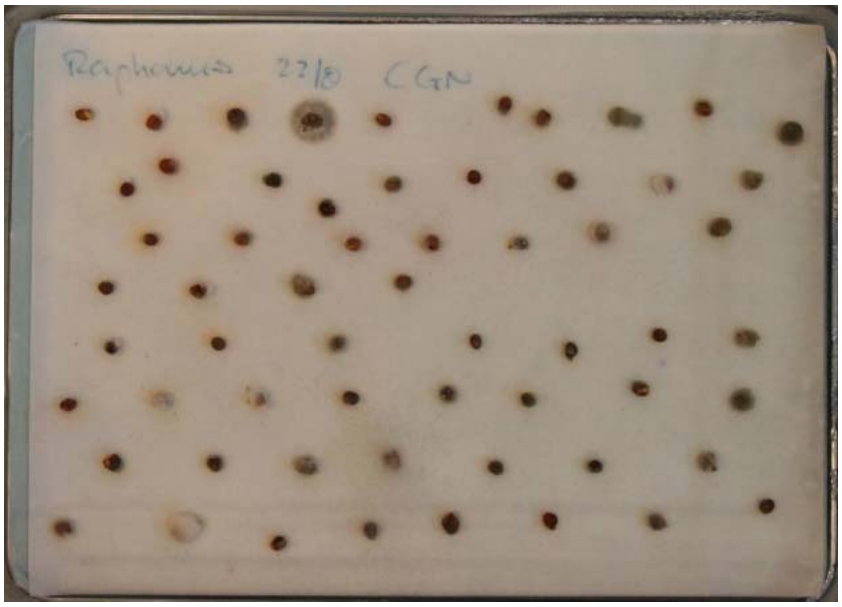

Figure 5. *Seed health test on a* Raphanus sativus *seed lot, showing contamination with Alternaria type fungi. The seed lot was chosen for examination because it gave only 71% normal seedlings in the germination test (see Annex II).*

- Chlorophyll fluorescence sorting was performed with a *Sinapis alba* seed lot that contained many seeds with a green appearance. In the low chlorophyll fluorescence fraction (the most mature seeds) still green seeds were observed, indicating that with those seeds the green color was not caused by residual chlorophyll. A germination test with the three fractions showed that all germinated fast and almost 100%. The chlorophyll fluorescence sorting had therefore no advantage for this seed lot.

Comments

- Upon harvest the seeds have a large range of maturity. Most of the full mature seeds are lost due to shattering. Consequently most seeds that are still in the siliques are unavoidably not full mature. Many seeds may have acquired maximum dry weight and therefore maximum seed size, but have not yet finalised maturation and acquired optimum stress tolerance. When the seeds are dried quickly after harvest, this situation is fixed. The sieves used during seed cleaning remove only the real immature and therefore smaller seeds. When the harvested inflorescences are dried more slowly (in environment protected from rain and high temperatures), then many of the seeds can more or less finalise there maturation drying in the siliques and

gain in quality. At seed companies it is also practice to dry the harvested horticultural *Brassica* seed crops slowly.

- Control of humidity at the end of seed development is important to avoid pre-harvest sprouting. With the glasshouse produced seeds this should be possible.
- Forced drying with heated air gives a high risk for deterioration.
- Intermediate storage of the harvest after drying is not controlled for humidity, this will result in non-wanted ageing of the seeds.
- Seed borne diseases are not uncommon with *Crucifereae* seed production, especially upon outdoor production. A common pathogen is the fungus *Alternaria brassisicola*. Also bacterium *Xanthomonas campestris p.v. campestris* (*Xcc*) infection is observed in some occasions. Observations on CGN seed lots showed indeed the presence of *Alternaria* type fungi in part of the seed lots.
- Research by PRI has shown that *Xcc* can be transmitted through (pollinating) insects. Because of the importance of *Xcc* infection in international transfer of seeds, it is very useful that the staff involved in seed production is well aware of the disease symptoms. Seed infection with *Alternaria* should also be checked.

Flax

The *Linum* (flax) collection contains five species, but 99% of the accessions is *Linum usitatissimum.* Flax flowers rather uniform. At some occasions lodging is observed. Foraging by birds may result in seed losses. Flax seeds do not shatter (not dehiscent) and can therefore be harvested when fully mature. The crop is harvested once the seeds can be heard moving in the capsule upon shaking. Seeds can remain some time on the plant in the field in the mature stage before harvesting. Pre-harvest sprouting has never been observed. Upon harvesting a bunch of stalks is cut. A bag is placed on top of the bunch, and the whole is place head down in a wooden crate. The material is often force dried at 25 °C directly after harvest. Subsequently the material is stored in the shed without temperature and humidity control for about half a year till cleaning of the seeds. After cleaning the seeds are stored at 15 °C and 15% RH. In general seed germination is rather high.

Diseases

No problems are encountered with seed borne diseases.

Comments

- Upon harvest the seeds are all full mature and already relatively dry. The harvest seems optimal.

Recommendations

- After drying a better controlled storage, especially at low relative humidity, should be considered.

Forage crops

The forage crops include two main groups, clovers (*Trifolium pretense* and *Trifolium repens*) and the grasses (*Lolium*, *Phleum*, *Dactylus*, *Poa*, *Festuca*, *Agrostis*). Seed production is in the open field.

Clovers (*Trifolium pretense* and *T. repens*)

Clover seed production is performed in isolated plots with about 70 plants. Flowering is over an extended period of time and mature seeds shatter. Pollination is by local insects. To get a more uniform flowering with red clover (*T. pretense*), the first flowers are removed by mowing the crop once. This is not performed with white clover (*T. repens*) accessions. The seeds are harvested by mowing the crop and putting the plant material in jute bags. This material contains pods from all maturity stages. Because of the abundant presence of vegetative material as leaves, the harvest is rather wet. The bags with material are directly dried by forced air at 25 °C. After the drying the material is stored for about half a year in the shed without humidity control till seed cleaning. After cleaning the seeds are stored at 15 °C and 15% RH.
Often soil is incorporated with the harvest. Upon cleaning of the seeds small clay particles with the size of seeds can get included, reducing seed purity. This gives problems upon automated counting of the seeds. Germination tests are performed between half and one year after harvest. In general seed germination figures are high.

Grasses

Seed propagation takes place with 70 plants per plot. The inflorescences of *Lolium* are fixed to prevent lodging. Flowering varies per accession and is related to the vernalisation status of the plants and can vary within a plot. The seed shatter at maturity. In July and August seeds are harvested before shattering by picking the inflorescence when the stalks are yellow and dry. It is assumed that nutrient transport to the seeds has stopped at that moment. Harvesting can be performed multiple times per plot. The *Dactylis glomerata* crop is harvested once the seeds in the upper half cm from the ear have been shed.
The harvest is put into jute bags and dried slowly indoors, without temperature control. After some time the harvest is dried further by forced air. Thereafter the dry material is stored in a closed compartment at 20 °C (large climate room), but without humidity control. Seed cleaning is performed after about half a year. After cleaning the seeds are stored at 15 °C and 15% RH.
Germination tests are performed between half and one year after harvest. With *Lolium* germination figures are mostly good, but for unknown reason it has been poor in one of the last years. *Dactylus* seed germination is in general not high, according to the seed companies a frequency of 70% is good for these seeds. Seeds from *Festuca* have in general a short longevity.

Diseases

With the clover and grass seed production no seed borne diseases have been observed.

Practical observations:

- A *Lolium perenne* seed lot that was examined contained some green seeds and some seeds with a black color below a partly translucent testa.
- Observations with a binocular microscope showed that a poor germinating *Lolium perenne* seed lot is most likely contaminated with *Claviceps purpurea* (ergot) (Figure 6).

Figure 6. Contamination of a Lolium perenne seed lot with most likely Claviceps purpurea (ergot).

Comments

- The clover seeds have a large range of maturity at harvest and the full mature seeds are lost due to shattering. Consequently most seeds that are harvested are not full mature. Many seeds may have acquired maximum dry weight and therefore maximum seed size, but have not yet finalised maturation and acquired optimum stress tolerance. When the seeds are dried quickly after harvest, as is done with clover, this situation is fixed. With the sieves used during seed cleaning only the real immature and therefore smaller seeds are removed. Although in general seed quality with clover is already high, further improvements on longevity can be expected upon slower drying. When the harvest is dried slowly (in environment protected from rain and high temperatures), than many of the seeds can more or less finalise there maturation drying in the siliques and gain in quality. However, care should be taken that initial drying is not too slow because of the large amount of moisture in the leaves.
- With grass seeds this modest drying is performed already.

Recommendations

- Dry the harvested clover not directly by heated forced air, but initially in a modest way by non-heated air and not too long. Subsequently store the harvest in the jute sacs, protected from rain and high temperatures, for one or two weeks. Thereafter, before fast drying in the drying cabinets with forced dry air is OK. However, care should be taken on the moisture coming from the leaves.
- After drying a better controlled storage is recommended for both the clover and grass crop. Especially storage at low relative humidity should be considered.
- There is no complete lack of seed borne diseases as expected. More control on this aspect is needed.

Fruit vegetables

Pepper (*Capsicum spp.*)

Seed production is performed under protected cultivation, in glasshouses on hydroponics (rock wool) culture. Seed production is for a part performed by Dutch seed companies and part of the collection is multiplied by CGN itself. Absence of virus infections during seed production is controlled by the Dutch Plant Inspection Service.
With *Capsicum* species the optimal harvest moment for the seeds is estimated per accession, by growing the first fruits till definitely (over)mature, with softening of the fruit. This gives an indication of the colour change during fruit maturation. It is assumed that seed maturation is parallel to fruit maturation. In general fruits (with seeds) are picked when the colour of the fruit changes to its mature stage (often red and absence of green parts) and/or when they start to soften. *Capsicum chinense* fruits remain firm, here the fruits are picked when they turn light-red.
Accessions with yellow fruit types are know to provide risks of germination within the fruit when fruits are harvested mature or a little later. Since it is not feasible to remove germinated seeds by hand, it is chosen to harvest seeds from those accessions a little premature, from fruits with still green parts. Hot pepper types contain capsaicin in their seeds, which is supposed to inhibit germination of the seeds in the fruit. Indeed germination of seeds in mature fruit of this pepper type has never been observed.
Blossom-end rot of the fruits may affect the quality of the seeds. When this occurs such fruits are discarded. If all fruits are affected, than seeds need to be extracted and the germination test will answer if quality is still acceptable.
Seeds are extracted from multiple fruits per accession. Since the number of fruits per harvest is limited, the picked fruits are stored for at most two weeks cool or occasionally at room temperature, till enough fruits are harvested from the accession to get an efficient seed cleaning. During this storage the fruits are checked for softening (very soft and wrinkled fruits are rejected).
Seed extraction is performed by removing the seeds by hand from large fruits, by squashing medium sized fruits with a large PVC block, or by squashing small fruits with the hand. Germination tests show no deleterious effect of the PVC squashing. The seeds are separated from fruit tissue using water, the seeds will sink to the bottom of the vessel and empty 'seeds' will float.
Seed companies normally use a one hour trisodium phosphate soak for seed separation instead of water, to destroy simultaneously potential viruses in the seeds. An additional advantage they observe, is that when a germination test is performed one or two weeks later, germination is very uniform. CGN staff is afraid that trisodium phosphate can harm the seed vigour and therefore do not use it and the seed companies that reproduce for CGN omit the use of trisodium phosphate for the CGN seeds. CGN advices their clients to give the seeds a trisodium phosphate sanitation treatment prior to sowing.
After extraction and the initial drying, the seeds are stored for about one year in paper bags at 15 °C and 15% RH till the germination data are available. Germination tests are performed by the Naktuinbouw. After positive germination results the seeds are packed and stored in the CGN collection

Lycopersicon spp. (tomato)

Seeds are produced in glasshouses on hydroponics culture as with the pepper collection. Also with this crop part of the seed production is presently done by the Dutch seed companies, according to a protocol set by CGN. At part of these companies the plants are monitored during seed production for the absence of *Clavibacter michiganensis*, but this is not performed at CGN and some other companies. At CGN and all companies the seed production is monitored by the Dutch Plant Inspection Service for absence of Pepinomosaic virus.
Ripe tomato fruits are cut in halves and the seeds with fruit juice are transferred to cups and diluted with an equal amount of 2% HCL solution to remove the fruit tissue. After 1 hour the seeds are washed in a sieve with water, and dried on filter paper at room temperature. After the initial drying, the seeds are stored for about one year in paper bags at 15 °C and 15% RH till the germination data are available. Germination tests are performed by the Naktuinbouw. After positive germination results the seeds are packed and stored in the CGN collection. In contrast to commercial practice, a trisodium phosphate treatment is not applied to the tomato seeds reproduced for CGN. No viviparous germination nor seed borne diseases have been encountered with tomato seed reproduction by CGN.

Eggplant (*Solanum spp.*)

Seed production is in glasshouses on hydroponics culture, as with the pepper collection. Here also part of the collection is reproduced by the seed companies. Absence of virus infections during seed production is controlled by the Dutch Plant Inspection Service. Mature fruits are picked and stored for at most one week at ambient temperature before seed extraction. The eggplant fruits are broken or cut in small blocks and squashed with a large PVC block to release the seeds. The seeds are washed and dried on filter paper at room temperature. After the initial drying, the seeds are stored for about one year in paper bags at 15 °C and 15% RH till the germination data are available. Germination tests are performed by the Naktuinbouw. After positive germination results the seeds are packed and stored in the CGN collection.
No viviparous germination nor seed borne diseases have been encountered with eggplant seed reproduction by CGN.

Cucumber (*Cucumis sativus*)

A large part of the collection is reproduced by the seed companies. Cucumber seeds are produced in glasshouses on hydroponics (rock wool) culture. For seed set manual pollination is performed and fruits are labelled with the date of pollination. Fruits are mature eight weeks after pollination, they change colour to yellow/brown. Seed maturation is considered synchronously with fruit maturation. Mature fruits are picked and placed for at most one week at the floor next to the mother plant. According to the seed company staff, this practice should increase germination of the harvested seeds. No information is available on the effect of this practice on longevity of the seeds. After these few days storage in the greenhouse, the fruits are stored for another few days at low or ambient temperature, varying between the producing company and if enough fruits of an accession are harvested for efficient seed cleaning. Cucumber fruits are cut in halves and the seeds with fruit juice are transferred to cups and diluted with an equal amount of 4% HCL solution to remove the fruit tissue. After 1 hour the seeds are washed in a sieve with water, and dried on filter paper at room temperature. After the initial drying, the seeds are heated for three days at 76 °C to remove potential viral diseases. Thereafter the seeds are stored for about one year in paper bags at 15 °C and 15% RH till the germination data are available. Germination tests are performed by the Naktuinbouw. After positive germination results the seeds are packed and stored in the CGN collection.
In some occasions germination of the seeds within the fruits (vivipary) has been observed. This has been noted in the logbook. Seed quality of the seed lots with these partly germinated seeds was too low for incorporation in the collection and a new production is planned. With other seed samples a strong level of dormancy was found. Initial germination test showed lack of germination but the seed remained hard. Cutting the tips of the seeds revealed that these seeds were viable. Dormancy was released during the four months storage at 15 °C and 15% RH .

Melon (*Cucumis melo*)

Melon accessions are in principle reproduced as cucumber seeds. To prevent potential infection with viruses, the seeds receive a dry heat treatment at 70 °C for three days.

Diseases

As mentioned above with the fruit vegetable crops no problems are encountered with seed borne diseases. During seed production, the plants are checked for the absence of viral diseases and the cucumber and melon seeds receive a dry heat treatment to destroy viruses potentially present in the seed. Pepper, tomato and eggplant seeds receive no antiviral treatment. Tomato seed production is monitored during some productions for the absence of *Clavibacter michiganensis.*

Comments

- With the fruit vegetables it is often clear when the fruit is mature and seeds are assumed to mature synchronously. Therefore risks of harvesting less mature seeds are for most of the accessions rather low. An exception is *Capsicum* with yellow fruits. These seeds are deliberately harvested less mature to prevent onset of germination processes prior to harvest. These seeds may provide risks of a relative faster loss of longevity. It is interesting to compare germination data soon after harvest and after some year storage from red and yellow fruited *Capsicum* annuum accessions to analyse if these seeds differ in longevity.
- Prior to radicle protrusion the onset of germination processes can be analysed non-destructive with *Capsicum* seeds by analysis of chlorophyll fluorescence. This method might have potential for predicting relative short longevity of potentially pre-germinated seeds in certain seed lots.
- The seeds are dried soon after harvest, initially at room temperature and subsequently at 15 % RH and 15 °C. Therefore, the seeds are stored almost immediately at optimal temperature and moisture conditions.
- It is remarkable that the cucumber seeds can withstand a three days dry treatment at 76 °C, without apparent loss of germination capacity. However, the long term effect on seed longevity is not known. Since not all cucumber seeds reproduced in the past have received a heat treatment, CGN recommends their clients to apply an additional heat treatment before sowing the cucumber seeds. This raises the question if CGN should perform a heat treatment prior to storage, especially because of its potential risk of reducing subsequent seed longevity. It is advised to perform tests on the effect of the heat treatment on cucumber seed longevity. The accessions showing risks of viviparous germination can provide useful seed material for such experiments.

Recommendations

- Analysis of the longevity of seeds from yellow fruited *Capsicum* accessions should be compared with that from red fruited accessions, through analysis of germination data. This will provide more information on the question if seeds from yellow fruited accessions should be tested more frequently for viability loss.
- Potential of the chlorophyll fluorescence method to predict longevity in *Capsicum* seeds should be analysed with a few seed samples from yellow fruited accessions.
- The effect of the dry heat treatments, performed with cucumber and melon seeds, on longevity of the seeds should be analysed, to weigh the risks of virus infection against risks of viability loss and multiplication costs. Also international rules that allow only distribution of seeds after an antiviral or other sanitation treatment are important in this respect.

Grain legumes

The main grain legumes crop are faba bean (*Vicia faba*), pea (*Pisum sativum*) and two lupine (*Lupinus*) species. Seed production is done in the open field.

Vicia faba

The flowering period varies between two and eight weeks. Consequently seeds from different maturity are on the plant once the first seeds are mature. The seeds are enclosed in pods, which do not open, therefore seed shedding does not occur and the crop is harvested once the plants contain enough dry pods with a yellow or brown colour. Problems are only encountered when it rains a lot in the period before harvest. The plants from a single accession are harvested in one or two times. The crop is dried directly after harvest by force drying at 25 °C. Subsequently the material is stored several months indoors, without control of humidity. In December or January the pods are threshed and the seeds are cleaned using sieves. Mechanical damage due to the threshing hardly occurs. Seeds with diverging colours, that can indicate poor seed quality, are removed by hand. Germination frequencies are always high. No problem is encountered with hard seed coats delaying or inhibiting germination. It is frequently difficult to obtain enough seeds because of low seed set and often the seed production has to be repeated because of too low seed production.

Pisum sativum

The plants have a very extended period of flowering. Therefore the pods with seeds are hand harvested once or twice a week, when they have a dry appearance. The pods are put in a jute bag and dried with forced air at 25 °C. Thereafter the material is stored indoors, without humidity control, for about half a year till seed cleaning.

Lupinus

Also lupine plants have an extended period of flowering, but here combined with shattering of the mature seeds. Therefore lupine seeds are harvested prior to full maturity. The inflorescences are harvested and the part with green pods is removed. The harvest is once or twice for a single plot. The inflorescences are packed in jute bags and immediately dried by forced air at about 25 °C. Thereafter the material is stored indoors without humidity control for about half a year till seed cleaning.

Diseases

During the growing season the plots are both visually and by means of serological tests monitored for Pea Seed-borne Mosaic Virus (PSMV). Plants detected with PSMV infections are removed entirely. No problems are encountered with seed borne fungal diseases.

Comments

- The Pea and especially the lupine pods will contain less mature seeds during harvest. Heated force drying immediately after harvest may result in reduced seed longevity, especially when the temperature is not correct.
- Storage of the crop after drying is non-controlled and for several months, this may result in accelerated ageing of the seeds, especially in periods when relative humidity is high.

Recommendations

- Dry the pea and the lupine pods initially more slowly, for about a week, thereafter forced air drying can be performed.
- After drying a better controlled storage, especially at low relative humidity, should be considered.

Leafy vegetables

The main leafy vegetable crops for CGN are lettuce, spinach and their wild relatives. Seed production is done in glasshouses.

Lettuce and relatives

The plants flower during an extended period and especially for wild accessions the period can be very long. Consequently, once the first seeds are mature the plants have seeds from different maturity. For the wild accessions the seeds are shed after maturation. For some cultivated accessions the mature seeds remain enclosed in the calyx. To capture the released seeds, the inflorescences are loosely packed in perforated plastic. When enough seeds have been produced, either shed or still in the calyx, the inflorescence is cut from the plant. This harvesting is in August, about six weeks after onset of seed shedding. The harvested material, still enclosed in the perforated plastic, is put in a paper bag. The next day the bags with inflorescences and seeds are placed in drying cabinets with forced air drying at temperature of 25 °C for several days. Dried seeds and inflorescences are subsequently stored in the seed storage facility of PRI at 13 °C and 30% RH, until the seeds can be isolated and cleaned. Cleaning is performed in November or December with a 'blower'. After cleaning the seeds are stored at 15 ºC and 15% RH, till the germination data are available and seeds can be packed and put in the collection.
Germination frequencies are generally high, although clear quality differences are observed between seed production years.

Spinach

As with lettuce, also spinach flowers over an extended period of time. However the spinach seeds are not shed. After flowering and seed development the mother plant gets yellow and dry. Harvest is performed in July and August. Once the inflorescences are more or less dry, they are cut from the mother plant and put in paper bags. The material is dried directly after harvest with forced air at 25 °C and subsequently stored at 13 °C and 30% RH till cleaning. Cleaning is performed in October or November and cleaned seeds are stored at 15 °C and 15% RH for about one year till the germination data are available.
According to experience at one of the seed companies spinach seed quality is damaged when temperature during drying reaches 30 °C. Although this can not be confirmed by reports in the scientific literature, it is worthwhile to avoid drying at temperatures above 28 °C. There seems no check if the drying is too short or too long.

Seed production by third parties

Seed production for the CGN lettuce and spinach collection is also performed by seed companies. CGN has no control on the harvest, drying and storage conditions after harvest. Frequently damaged seeds are found in the spinach seed lots and additional cleaning is needed. This damage is likely a result from the threshing.

Diseases

No problems are encountered with seed borne diseases. Lettuce seed production is screened against the presence of lettuces mosaic virus by the Naktuinbouw.

Remarks

At IVT (one of the predecessors from PRI and from which CGN took over the responsibility for the lettuce collection) seeds were stored in glass jars above dehydrated silica gel. Whenever the silica gel turned from blue to pink, it was refreshed. This method of storage was performed from 1973 till 1986 when CGN took over the responsibility. In 1986 seeds were equilibrated at 15 °C and 15% RH and packed in foil laminated bags. Many of these once ultra-dry stored seeds still have a high germination capacity.
At a visit to the forced drying cabinet, it appeared out that the temperature was several degrees higher than described in the protocol. It was not clear who is responsible for setting the temperature in the cabinet. Also it was

not clear for how long the seeds remained in the drying cabinet. The impression was that this could last a few weeks. As mentioned before, the higher temperature can have a negative effect during drying itself, but also prolonged storage at these higher temperatures should be avoided.

Comments

- Lack of adequate temperature control during drying provides a threat for the quality of the seeds.

Recommendations

- More control on the temperature in the drying cabinet after harvest is needed. Registration through a logbook, minimum-maximum thermometer or temperature data logger can be considered. Also the period that the harvest is in the drying cabinets should be registered. Both will aid in increasing the awareness of the staff for potential factors that can have a negative influence on seed longevity.

Onion and leek

Seed production for onion and leek is both protected from rain in plastic covered tunnels or in glasshouses. There is some variation in flowering between individual plants. Harvest is therefore performed twice a week over a period of several weeks in July and August. The upper part of the flower stalk, containing the fruits and seeds is harvested once the first fruits start releasing the seeds. The harvested material is put in linen sacs and left in the tunnel or glasshouse, where it slowly dries, till all material is harvested. During this drying the material is protected from direct sunlight. In 2006 the material was left in the glasshouse for a relatively long period, while temperatures in the glasshouse had risen above 35 °C during the day. However, there was no apparent loss of germination capacity. After the initial drying in the tunnel or glasshouse, the material is further dried in the drying cabinets with forced air at 25 °C. After drying the material is stored in the PRI seed storage facility at 13 °C and 30% RH for preferably no longer than a few months. The seeds are cleaned in December, with a blower and clipper. After cleaning the seeds are stored at 15 °C and 15% RH, till the germination data are available and seeds can be packed and put in the collection. Seeds from wild accessions may possess dormancy.

Diseases

No problems are encountered with seed borne diseases.

Comments

- Seed production and drying is rather optimal, since seeds are harvested almost mature and care is taken to let the less mature seeds finalise the maturation process before drying to lower moisture contents.
- Forced heated drying provides risks when the temperature is not adequately controlled.

Recommendations

- A log book on seed handling, with special attention to temperature can be useful.

Potato

The potato accessions vary in time of flowering and the moment the berries are mature. There is also variation in length of flowering. In general, the accessions are harvested only once, when the berries can easily be picked from the plant. In some occasions berries drop from the plant earlier. When this is observed, the berries are also collected and stored at 5 °C for several weeks. In the autumn the berries are transferred from the cold to room temperature to allow 'maturation' of the fruits and seeds. Once most berries of an accession are getting soft, the seeds are removed from the berries, mostly by squeezing the seeds from the fruits in water. Often the empty seeds will float and are washed away with the debris. Seeds are dried on paper for a few days at room temperature. Some additional cleaning can be performed with the use of a blower. The cleaned seeds are first stored in the PRI seed storage facility at 13 °C and 30% RH. When all accessions have been cleaned and dried the seeds are transferred to paper bags and stored at 15 °C and 15% RH.
Because of dormancy in most potato seeds, the germination test is performed about a year after harvest. Frequently relative low germination frequencies are observed. Seed productions with low germination rates are not included in the collection and new seed production is advised. However, the low germination frequency can very well be because of residual dormancy, despite the use of GA3 in the germination test. Improvement of the germination test to show the frequency of all viable seeds is highly needed.
Most potato seeds have dormancy and therefore no problem with pre-harvest sprouting or pre-germination before harvest. An exception is *Solanum phureja*, which lacks both tuber and seed dormancy. It has once been noted upon cleaning of *S. phureja* seeds that part of the seeds had germinated either in the fruit, or in response to the wet cleaning. Even when no germinated seeds are noticed, the seeds from this species can exhibit onset of germination and therefore a reduction in longevity. Germination is tested every ten years.

Diseases

During seed production the plants are screened by the Dutch Plant Inspection Service for absence of several virus diseases and one viroid disease. No problems are encountered with fungal or bacterial seed borne diseases.

Comments

- Seeds are mostly harvested near-mature and are allowed to mature in the berries separated from the mother plant. For logistic reasons this seems the most optimal. The berries with the seeds are stored for a considerable period, during which the matured seeds are in a wet environment. Without dormancy this should give high risks for onset of germination prior to harvest or even pre-harvest sprouting. Because of high levels of dormancy with almost all accessions this is no problem. However, more care should be taken with accessions that lack dormancy. At present no special precautions are taken with seed production and cleaning of low dormancy seeds, neither is the viability tested in shorter intervals. It is worthwhile to look at germination data for seeds from this species, after storage, in comparison to seeds from other potato species.
- When the germination frequency is low, an advise to make a new seed production is only needed when lack of germination is due to poor seed quality and not to dormancy. A new seed production will cost a lot of budget, and also other accessions need to be reproduced. Moreover the seeds are not available for CGN clients when they are not incorporated in the collection. More detailed observations of the potato seed germination are needed when low germination frequencies are observed.

Suggestions

- Accessions that lack development of seed dormancy should be identified. Lack of tuber dormancy may indeed be a good indicator. Here special attention is needed with the seed harvest. If possible pick the berries near mature or mature, but not over mature (soft). Store the berries cold and remove the seeds without subsequent storage of the berries at warm temperature. Dry the seeds quickly after removal (and cleaning) from the berries. Examine the seeds carefully (e.g. using a binocular microscope) for lack of pre-harvest sprouting.
- Put more emphasis on the germination test when low germination frequencies are observed, to identify if poor germination is due to low seed quality or to dormancy. An improved dormancy breaking protocol may be developed. Alternatively, the endosperm cap opposing the radicle tip may be cut from non-germinating seeds.

Appendix II Observations on a selection of CGN samples

To improve the insight in seed quality of CGN produced seeds, a number of seed samples from different crops were examined morphologically, using a binocular microscope. The choice of the samples was based on observed poor germination with some samples and comparison with other samples produced in the same year. The sampling was not intended to be representative.
AS = abnormal seedlings, NS = normal seedlings

Sample Identity	Species	Observations on the dry seeds	Germination data % (NAK)
7302	*Raphanus sativus*	Contains also green seeds, seeds with gray testa and seeds with black spots. A seed health test showed that most gray seeds were contaminated with fungi.	NS 71 AS 23 Other 6
030610	*Raphanus sativus*	Sample contains a few germinated seeds, some green seeds, some black seeds.	Low
23812 /990735	*Brassica oleracea*	Sample contains a few germinated seeds.	Good
11979	*Sinapis alba*	Sample contains several germinated seeds, many green seeds, which is very clear due to the light colored testa. Seeds were sorted into fractions with low, medium or high chlorophyll fluorescence levels. A germination test showed that seeds from all three fractions germinated very rapid and for about 100%.	NS 88
11976	*Sinapis alba*	Sample contains a few germinated seeds, many green seeds as the previous.	
070012	*Eruca sativa*	Seeds have a healthy appearance, but many green seeds.	NS 33 dead 66
11968	*Sinapis alba*	Sample contains a few germinated seeds, about one quarter of the seeds is gray, some even black. A seed health test showed that most gray seeds were contaminated with fungi.	NS 86
11963	*Raphanus sativus*	No germinated seeds, some gray seeds, healthy looking seeds, some broken seeds.	NS 82
7099	*Brassica oleracea*	Sample contains a few germinated seed, most seeds shrivelled.	NS 84 AS 13 Dead 3
10023	*Lolium perenne*	Sample contains a few green seeds, some seeds black below partly translucent testa.	NS 72
9991	*Lolium perenne*	Contains ergot (*Claviceps purpurea*), some seeds with appearance of fungal growth.	NS 46
000004	*Dactylus glomerata*	Some seeds are green, no clear abnormalities.	NS 16
8382	*Triticum turgidum*	All seeds are shriveled, one seeds germinated, some seeds have appearance of fungal growth, some seed are light pink. A seed health test showed abundant presence of Fusarium infection on the seeds.	NS 26
8035	*Triticum aestivum*	Many if not most seeds have sprouted.	?
8033	*Triticum aetivum*	Many if not most seeds have sprouted.	?