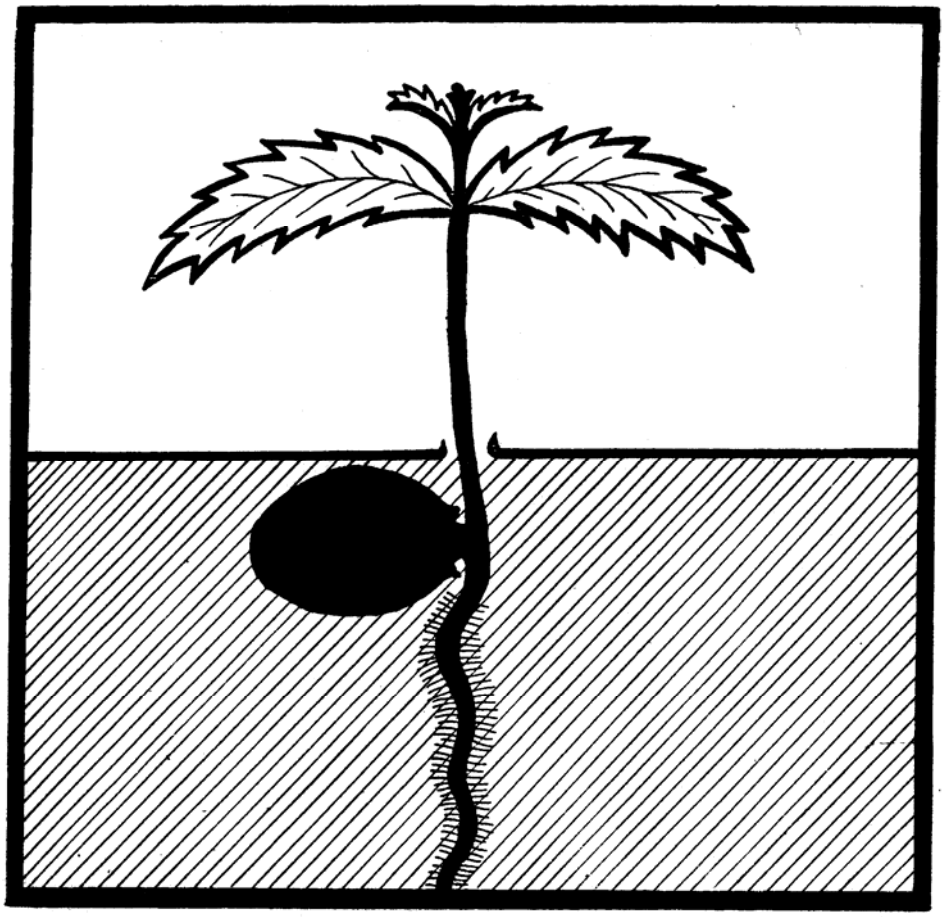
NR Study-note F120e

SIMPLE GERMINATION TESTING



Based on text and graphics in:
TREE SEED HANDLING:
A manual for field staff in Nepal.
Field Document No. 11
HMG/EEC/ODA National Tree Seed Project
HMG/UNDP/FAO Community Forestry Development Project
by A.M.J.Robbins and N.B.Shrestha (1986)

Revised July 2004

PREFACE to the original document

Obtaining adequate supplies of high quality, healthy seeds for the Community Forestry Project is a recurring problem. This is made more difficult as we use astmany as 80 different species to suit the ecological requirements of the various sites, as well as to meet the preferences of the farmers who need tree species for fodder, fuel or multipurposes.
The original nursery manual published as a Field Document (No. 2b) devoted only one chapter to seed collection and the subject was. treated only in a very general manner. The present document has treated the subject of "Seeds" much more thoroughly with detailed guidelines for seed collection, seed processing and treatment, rules for seed storage and finally testing the seed that has been collected. It is primarily meant for those involved in reafforestation and afforestation. The document is well illustrated with sketches and written in both Nepali and .English making it easier to comprehend. It is highly commendable that complex scientific information about various aspects of seeds have been discussed in such plain and simple manner.
Seeds are the most essential basic resource material for raising successful plantations. Better seeds grow into better seedlings which ultimately, will grow into healthy trees. Therefore, this document will go a long way in solving practical difficulties in seed collection i.-e. from tree climbing and harvesting to seed storage and distribution and will encourage field staff to collect quality seeds to ensure good quality plantations.
We are appreciative and also indebted to Mr. Marcus Robbins, Silviculturist, ODA and-Mr. Narendra Bahadur Shrestha, Chief, Afforestation Unit, CFAD for preparing such valuable guidelines. We are sure that all concerned will find it purposeful and of practical utility.

M. S. Ranatunga, Chief Technical Advisor, Community Forestry Development,Project
B. P. Kayastha, Chief, Community Forestry and Afforestation Division

INTRODUCTION to the original document

Afforestation in Nepal is currently around 15 thousand hectares per year, equivalent to 30 million saplings. This production requires at least 150 million viable seeds, equivalent to 15 tonnes of seed per year or 150 tonnes of fruit. These amounts will double within 5 years.
In view of this demand for seed, HMG/N has agreed to start a National Tree Seed Project (NTSP), with assistance from the European Economic Community (EEC) and the UK Overseas Development Administration (ODA), with the object of ensuring adequate supplies of high quality seed for all programmes of reforestation within the Kingdom of Nepal. The NTSP is based at the national Tree Seed Unit facilities of the Community Forestry and Afforestation Division (CFAD), which were established in 1981 under the Nepal Australia Forestry Project.
The geography of Nepal means that it is neither practical nor advisable to collect and distribute such quantities of seed as a centralised operation, and therefore each forest district or project must endeavour to become self-sufficient in seed supplies as far as is possible. The strategy of the NTSP is, therefore, to provide support to the districts in achieving this self-sufficiency, and to take responsibility for aspects of seed supply that the district cannot handle.
As a first step in providing such support, this manual has been written for District Forest Controllers and their staff, with the aim of ensuring that proper seed handling practices are used in each forest district. The manual was originally written as 4 separate technical leaflets which have been put together under one cover. The manual covers general techniques only, and detailed handling of individual species will be published by the NTSP as separate leaflets.
The authors are very grateful for the help of many people in the preparation of this guide, in particular to: Mr. B. P. Kayastha and Mr. M, S. Ranatunga for their suggestion and support in producing the manual as a field document of the Community Forestry Development Project; the staff of the Forestry Research Project for their invaluable help in commenting on the text; Mr. Debendra Amatya (Forestry Services) for his willing assistance in translation into Nepali; and to Secretarial Support Services for arranging publication.
Readers who require further information, or have any comments or queries about the manual, are asked to write to the authors at the CFAD, Hattisar, Kathmandu.

A. M. J. Bobbins, (Technical Advisor, ODA)
N. B. Shrestha, (Chief, Afforestation Unit CFAD)

NOTE on the current publication

The fourth leaflet of the original manual has been reformatted here, in electronic form, with some modifications, as a follow-up to a study commissioned by FAO, to make tree seed extension literature more widely available. I am very grateful to Pierre Sigaud at FAO for his original initiative and support in doing this. The current version is one in a series of NR Study-notes produced by the author, for use in training courses.

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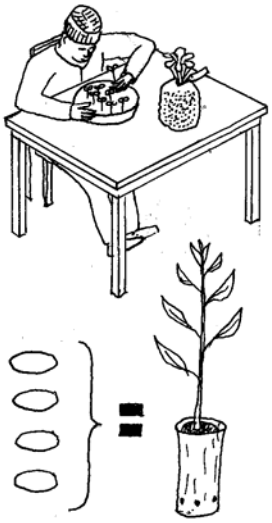
July 2004

VERSION July 2004

1 WHY TEST?

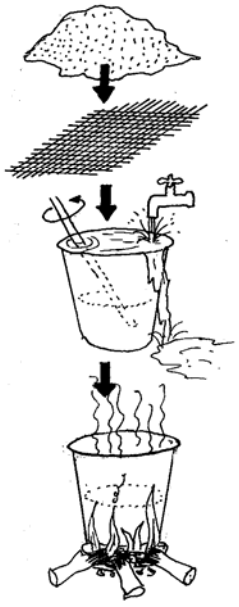
1.1 A lot of time, effort and seeds can be wasted in the nursery if seeds are sown without knowing how many are viable and have the potential of producing a seedling. A guess can be made from past experience, but collection, processing and storage methods can cause large differences between seed lots.



1.2 It is therefore important to test each seed lot for viability after collection, and also before sowing if some period of storage has been needed. By doing this, field staff can keep a check on whether their storage and nursery techniques are adequate or not, and take steps to improve them and save seed.

1.3 A test should be done under the best possible conditions for germination of the seeds, although these may not be possible in the nursery. Experience will show how many viable seeds will normally be required to produce one healthy seedling in the nursery. It should not be more than 3-4 viable seeds per seedling, but unfortunately this ratio is much higher in many nurseries.

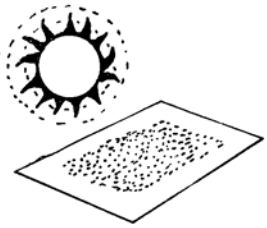
1.4 All seed lots distributed by a National Tree Seed Centre will be tested in the laboratory there, but lots collected locally or stored for some time after receiving them will need to be tested by field staff. A simple method of doing this is described here.



2 WHAT TO USE

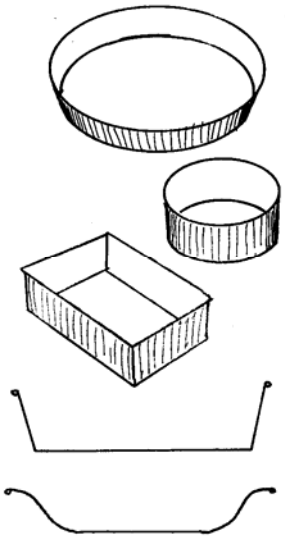
2.1 Sand substrate

Sand is the best substrate for general testing, but must be properly graded and sterilised. Prepare in bulk as follows: Obtain fine river sand, and sieve through fly screening (1 - 2 mm aperture) to remove large material. Place the sieved sand in a bucket (half full) or similar container, and wash out the fine silt. Do this by running water into the bucket and allowing it to overflow for 10 minutes or so, gently stirring the sand all the time.



2.2 Sterilising is important!

Drain off the free water and sterilise the damp sand by heating over a fire and steaming for one hour. Remove the sand and place in a thin layer on a clean surface (eg. polythene sheet) until fully dry, then store in a suitable container with a lid.



2.3 Water:

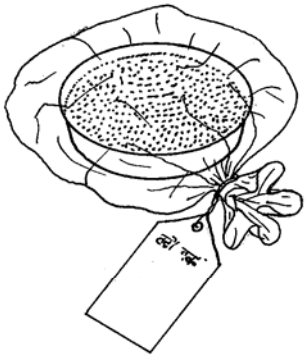
Unless very contaminated, running *water* can be used direct. Otherwise use boiled: filtered water prepared for drinking.

2.4 Container:

This should be large enough for 50-100 seeds of the species to be tested with a minimum ofoneseed[1]s width between seeds and should have sufficient depth to allow some development of the seedling. A lid is not necessary. The container can be made of clay, plastic metal or glass provided it can be easily cleaned It should have no holes in it. See note 1 for suggestions and sizes)

2.5 Sterilising the container:

When reusing the container, it is advisable to sterilise it. Metal, clay and glass containers can be sterilised by putting into water and bringing to the boil for 20 minutes. Plastic containers may be deformed by the heat, and should be soaked overnight in a dilute solution of bleach or disinfectant, or wiped with alcohol.



2.6 Plastic bag:

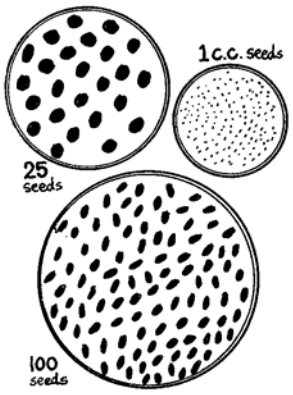
Each container will need to be placed in a thin, transparent plastic bag just larger than the container, so as to keep in the moisture. The bag is best closed with a piece of copper wire (1 mm diam.) as used by electricians, otherwise use string or rubber band.

2.7 Labelling:

Each bag should be labelled with a piece of cardboard, on which to write the name of the species, its identity number, and also the results of the test.

2.8 Avoid contamination:

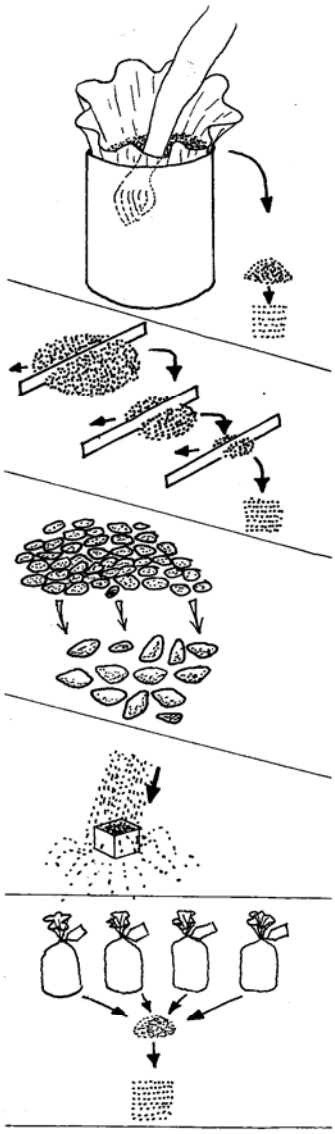
To avoid contamination of tests, it is best to use each bag once only.



3 HOW TO PREPARE THE TEST

3.1 Amount 'of seed to use:

Each test should be done on 100 seed (if about the size of pine or smaller), or else on 50 or 25, depending on how many will fit into the container. Very small seed (eg. Eucalyptus or Utis) should be sown by volume. About one cubic centimetre (1 cc) or less should do. (see note 2)



3.2 Sampling:

The seed used for the test must represent the whole seed lot. To make sure of this, mix the seed lot well, and take 1 or more fist-fulls from the middle of the lot, such that you have at least 500 seed. From this sample, count out 100 seed (or less if big) and return the rest to the seed lot.

3.3 Small lots:

If the seed lot is small, sample by pouring it onto a sheet of paper, and divide in half using a ruler. Take one of the halves and divide this in half. Keep on doing this until you have about 500 seed, then count.

3.4 How to count:

When counting the seeds, make sure you don't choose the best. Take the seeds just as they come from the pile you are counting from. If they are large seed, close your eyes while counting out the seed. It is very important that all types of seed are represented by the sample, and they are in the right proportions.

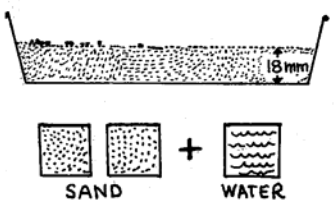
3.5 Use volume for small seeds:

When sampling very small seed that are tested by volume, sample as described above, but instead of getting 500 seed, get 5 times the volume you wish to sow. Then pour this over the container used for measuring volume, until the right amount is obtained.

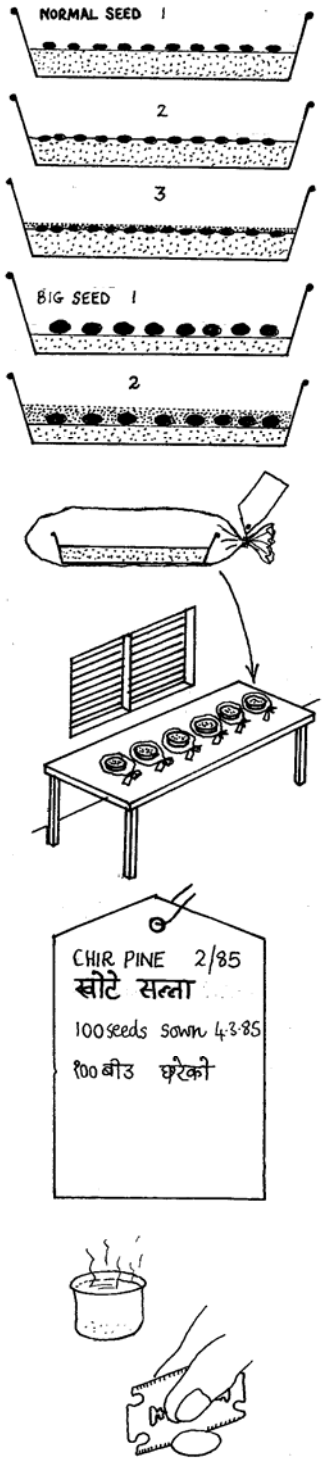
3.6 Sampling from several containers:

If seed is kept in several containers, take samples from all the containers and mix together before counting or measuring the seed for the test. If you think one container has seed of different quality to the rest (although part of the same seed lot), then you should do a separate test for that container.

3.7 Preparing the testing container:



Each test will require one container. Fill each container with sufficient prepared sand so that there is a layer about 3/4" deep (18 mm). Add enough water to dampen the sand, but not saturate it. As a guide, try adding a volume of water equivalent to 1/3 - 1/2 the volume of sand. Stir until uniformly moist, then press down and level the sand within the container with a wooden scraper.



3.8 Sowing the seed:

Place each seed individually on the sand, uniformly spaced, with at least one seed's width between seeds. Then gently press the seeds into the surface of the sand until level with the top of the sand. Just cover by sprinkling on some dry sand. If the seed is very large, it is better to put part of the sand, already moistened, in the container, then place the seeds in position and put the remainder of the moist sand around the seeds just covering them. Very small seed measured by volume can be sprinkled on with a spoon, and then covered with enough sand to hide the seed.

3.9 Where to put the containers:

Each container should be put into a polythene bag, which is closed with a twist of copper wire, elastic band, or string. The bag will let in light, but keep the container moist, so that further addition of water is not needed. Place the container in its bag indoors near a window, but out of direct sunlight. If the test is done in winter when it is cold, then keep the containers in the warmest room available.

3.10 Don't forget to label:

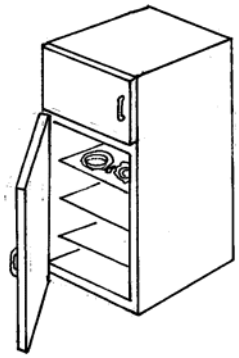
Label each bag with a piece of cardboard attached to the wire or string. Write on the label the species name, and any details, such as the identity number, so that it is clear which lot is being tested. Note down also the number of seeds sown and the date of sowing. The label will serve for recording and germination results as described later.

3.11 Treating the seed:

Some seeds may need to be treated before sowing in the nursery to hasten their germination. In this case, the seeds used for the test should receive the same treatment before testing, provided you know that the treatment does not damage the seed. (eg. hot water treatment of hard coated seed). (see note 3)

3.12 Be cautious:

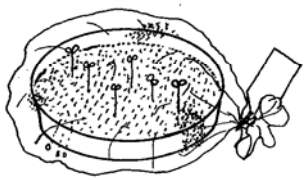
If in doubt about treating hard coated seeds, the seeds for testing should be individually chipped with nail clippers so as to make a small hole in the seed coat. This must be done at the end away from the embryo radicle.



3.13 Breaking dormancy:

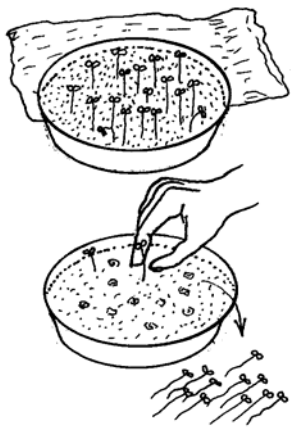
Seeds of species from higher altitudes may need to be stored for a period while cold and moist so as to break dormancy. In this case, the container and its polythene bag should be put somewhere cold, if possible in the lower compartment of a refrigerator, and left for several weeks before putting in a warm place for germination. (see note 4)

4 WHEN AND HOW TO MEASURE THE TEST



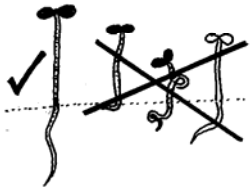
4.1 Checking and counting:

After a few days, check each test by removing the container carefully from the bag. If water has accumulated on the inside of the bag, pour it back into the container down one side. When a fair number of seeds (say 10) have germinated and properly emerged as healthy seedlings, carefully uproot them without disturbing other seed, and remove with the fingers or tweezers. Note the number of seedlings removed and the date on the label. Immediately afterwards, replace the container in its bag and close. Do the assessment one container at a time so as not to muddle up the labels.



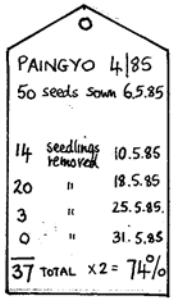
4.2 Keep on checking and counting:

Repeat thereafter every week or so, removing the new seedlings and noting the number and date until no further seedlings germinate. If any seed becomes mouldy, remove it so that it will not infect others. If there are a lot of seed ungerminated after a month or so, remove them and cut open with a razor blade. If the contents look healthy (white and firm) then the seed may be viable, but in need of some treatment before sowing.



4.3 Abnormal seedlings

If a seed germinates, but the seedling does not look normal (eg. small radicle, no chlorophyll (i.e. white rather than green), stunted, etc.) do not count it as a healthy seedling, but note its abnormality on the label. Such seedlings will probably not develop under nursery conditions.

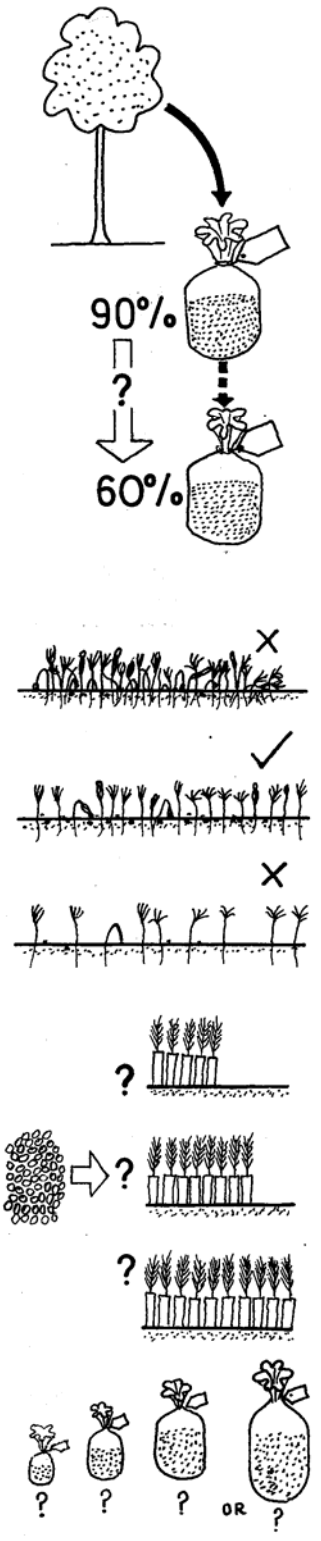


4.4 Calculating the germination percentage:

This is calculated by adding up the number of seedlings removed, as noted on the label. If 100 seeds were used, then the total is equivalent to the germination percentage; if 50 seeds were used, multiply by 2 to give the percentage; if 25 were used, multiply by 4. (see note 5 and 6)

4.5 Small seeds by volume:

In the case of small seed measured by volume, express the result as the number of germinated seeds per unit volume (eg. seedlings per 1 cc).



5 HOW TO USE THE RESULTS

If the tests are properly carried out and the results correctly calculated, the germination value can be useful in several ways.

5.1 Checking loss in Quality:

If you already have a figure for the germination value, but suspect that the seed has lost quality because of poor storage conditions, do a test and compare the results. For example, if a seed lot of Chir pine had 90% germination after collection, but gave 70% after 6 months storage, storage conditions would be poor and need to be improved. Actual losses in quality will vary depending on species.

5.2 Calculating sowing rate:

The germination value will enable you to calculate how much seed to sow to produce a given number of seedlings in the seed bed, and avoid sowing too densely or too sparsely. Correct sowing density will produce better seedlings that are easily transplanted. If the seed is sown direct into containers, the proper number of seed to be placed in a container can be calculated, so that most have a seedling but there is no wastage of seedlings.

5.3 Checking nursery techniques:

The test gives ideal conditions for germination which one cannot expect to copy in the nursery. However, it will enable you to calculate how many viable seed are needed to produce one healthy seedling in the nursery. With good techniques, you should not need more than 4 viable seeds to produce 1 seedling. If more are needed, the techniques should be improved. Remember that seed costs a lot of time and money to collect, and should not be wasted.

5.4 Ordering seed:

When you have a good idea of germination values, and the number of viable seed required to produce one seedling, then you are in a better position to order the right amount of seed for sowing. When this information is not available, there is a tendency to order too much, 'just in case' and often the excess seed is wasted.

5.5 Central testing:

If you are in doubt as to the quality of a seed lot, then send a sample to the National Tree Seed Centre. The Centre will have facilities for testing to a high standard which is not possible in the districts.

EXPLANATORY NOTES:

NOTE 1:

There are many locally available containers that are suitable for testing. They should be about 1.5-2.5" (35-60 mm) deep, with fairly straight sides. They can be square, rectangular or circular in shape. A suitable size for most species has a base area of about 15-30 sq." (90-180 sq. cm). This is equivalent to a dish of 4-6" (10-15 cm) diameter, or a rectangular box of 4x6" (lOx15 cm) or 5x5" (12 x 12 cm). Larger containers will do, and can be used for two tests if required. A very suitable contain- er is an aluminium dish with sloping sides as commonly found in the bazaar

NOTE 2:

The exact size of the measure is not important, provided that it can be related to the measure used for sowing in the nursery. The simplest way of doing this is to make box measures from cardboard and tape. For many small seeded species, 1 cubic centimetre or less is adequate for a test, but you will need larger (10, 100, 1000 cc) for nursery sowing. The following table gives the length of one side of a cube that will give these volumes:

Length of one side	**Volume of cube**
1 centimetre	1 cubic centimetre
2.15 centimetres	10 cubic centimetres
4.64 centimetres	100 cubic centimetres
10 centimetres	1000 cubic centimetres (or 1 litre)

These measures can be used to mark off volumes in other more durable containers, such as tins, bottles. You may also find these containers useful for larger seeds that can be counted, so that you can relate number of seeds to volume for these too. This way, you will not need to weigh seeds.
As a very rough guide, the number of seeds per kg can be converted to the number of seeds per litre (1000 cc) by multiplying using the following factors:

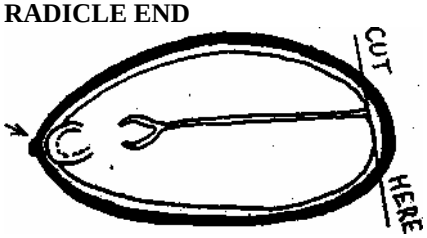
- Clean, dry seeds of most species X 0.5
- Seeds with thick coats or stones, X 0.6-0.7 (eg. Paingyo, Siris)
- Seeds with wings and fruit bits X 0.1-0.2 (eg. Sauer, Utis)

Eg. Chir pine will have 9000 x 0.5 = 4500 seeds/litre.
Check these factors by doing your own counts.

NOTE 3:

Before treating seeds by chipping, cut a few seeds in two and try to find the radicle. It will generally be at the end of the seed which was attached to the fruit and is marked by a round scar. Cut the opposite end. A typical hard-coated seed is drawn below:

RADICLE END



NOTE 4:

See note 6 of Technical Note 2 for species that need cold treatment.

NOTE 5:

Example using *Pinus roxburghii* (Chir Pine)
A sample of 100 seeds is tested, and the results are as follows:

Date	**Seedlings removed**
10/6	10
15/6	40
21/6	30
28/6	0
TOTAL	80

From the results, 80 out of 100 seeds germinated, therefore the germination percentage is 80%. (If 50 seeds had been used, and 40 germinated, then the germination percentage would be 40 x 2 = 80%.)
Chir pine has about 10,000 cleaned seed per kilogram, therefore the no. of viable seed will be:

10,000 x 80/100 viable seeds/kg.

The recommended density of Chir pine seed- lings in a seedbed is about 2000 seedlings per sq. metre. If all the seed germinate and produce seedlings, then the amount of seed to sow is:

2000/8000 = 0.25 kg or 250 grams/sq.m.

Since conditions for germination in the nursery seedbed are not ideal, one must add about 10% more seed:

2000 + 200 = 2200 viable seed, or 250 + 25 = 275 grams/sq.m.

Of course, not all the 2000 seedlings that should emerge will survive until and during transplanting. One might expect 50% to survive, thus every 275 grams sown on 1 sq. of metre will probably give:

2000 x 50 / 100 = 1000 plants in pots.

Not all these plants in pots will be suitable for planting out. If 75% are suitable, then the number planted will be:

1000 x 75 / 100 = 750 plants

Thus for every 2200 viable seed sown, 750 plants are produced, which is equiavlent to 3 viable seeds per plant.

Example using *Alnus nepalensis*
A sample of 2 cubic centimetre was tested, and the results were as follows:

Date	Seedlings removed
5/7	120
10/7	280
15/7	0
TOTAL	400

Thus 2 cubic centimetres of seed give 400 seedlings, or 1 cubic centimetre gives 200 seedlings.
The recommended sowing rate for Utis in the nursery seedbed is 3000 seedlings per sq.m. Therefore, if all seeds germinate, the following amount of seed must be sown:

3000 / 200 = 15 cc/sq. m.

Allowances for mortality are made in the same way as for the example using Chir pine.

NOTE 6:

If you have sufficient time, seeds and containers, it is always advisable to carry out tests on 2 or more samples of 100, 50 or 25 seeds from one seed lot, each sown in a separate container. The germination value is then averaged from these 'replicates'. If the difference between the replicate results is small (up to 10%) then you can be confident that the average result is a good estimate of the seed lot germination. However, if there is a big difference (say 25%) between replicate results, then use the average with caution. It may be advisable to repeat the test.
eg. If the replicate results are 75% and 85% (a difference of 10), the average is 80% and will be fairly reliable. However, if the results were 65% and 95% (30 difference), the average is still 80% but may not be a good estimate.