SEED QUALITY CONTROL IN THE LABORATORY

Mirza Hasanuzzaman, PhD
Associate Professor
Department of Agronomy
Sher-e-Bangla Agricultural University
E-mail: mhzsauag@yahoo.com

Laboratory seed tests aim to provide accurate and reproducible guidance, rather than absolute answers or predictions. Viability, germination and vigour tests all produce results that are usually greater than, and at best equal to, how the seed will actually perform in the field. An appreciation of what viability, germination and vigour measure can help maximize the understanding of the planting value or storage potential of seed.

Seed sampling

Accurate seed testing to determine seed quality attributes such as germination, purity etc. are based on seed samples taken from bulk quantities of seed or bagged seed organized into "seed lots". Since the result of seed quality tests are only reliable if the tests were carried out on representative sample of the seed lot, sampling of seed lots must be done using prescribed systematic sampling techniques. Procedures and techniques must be followed to ensure the seed samples are representative of the entire seed lot and provide accurate information used in evaluation. The International Seed Testing Association (ISTA) has established regulations and procedures for sampling of seed.

Seed sampling and testing is part of the seed procurement process but it may also be used by emergency staff and local officials to verify the quality of seed before delivery to farmers or if the seed has been stored for several months.

Seed lot

A seed lot is a specific, identified quantity of seed whose purity and quality is homogenous throughout entire lot. The maximum size of seed lot prescribed for agricultural and horticultural seed is 20,000 kg. **Seed sampling:** The process of obtaining a seed sample of a size suitable for test in which the same constituents are present as in the seed lot and in the same proportions.

Types of sample

1. **Primary sample:** A primary sample is a small portion taken from one point in the lot or container or bag.
2. **Composite sample:** The composite sample is formed by combination and mixing all the primary samples taken from the lot or container or bag.
3. **Submitted sample:** A submitted sample is a sample submitted to testing station. It must be of at least the size specified in the International Rules of ISTA (1993) for submitted sample and may comprise either the whole or a sub-sample of the composite sample.
4. **Working sample:** The working sample is a sub-sample taken from the submitted sample in the laboratory, on which one of the seed qualities is done.
5. **Sub-sample:** A sub-sample is the portion of a sample obtained by reducing the sample using one of the sampling methods prescribed in the International Rules of ISTA (International Seed Testing Association) for sampling in the laboratory.

Sampling intensity in bulk lots

When sampling seed lots are in bulk the following number of primary samples to be taken-

Lot size (kg)	Minimum number of primary samples
Up to 50	3
51-500	5

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman

501-3000	One primary sample for each 300 kg but not less than a total number of five
3001-20000	One primary sample for each 500 kg but not less than a total number of ten
20001 and above	One primary sample for each 700 kg but not less than a total number of forty

Sampling intensity for seed lots in bag or container

For seed lots in bag (or other containers of similar capacity that are of uniform size) the following numbers of primary sample are the minimum requirement. Usually a 100 kg weight is taken as the basic unit and small containers are combined to form sampling units not exceeding this weight, e.g. 20 containers of 5 kg each.

No. of containers in the seed lot	Number of primary samples
1- 4 containers	3 primary samples from each container
5- 8 containers	2 primary samples from each container
9- 15 containers	1 primary samples from each container
16- 30 containers	15 primary samples in total from the seed lot
31- 59 containers	20 primary samples in total from the seed lot
60 or more containers	30 primary samples in total from the seed lot

To obtain a composite sample of a lot of seed kept in bag-

- For lots of six bags or less each bag should be sampled.
- For lots of more than six bags, sample five bags plus at least 10% of the number of bags in the lot. (Round off numbers with decimals to the nearest whole number raising o.5 to the next whole number.)
- Regardless of the lot size it is not necessary that more than 30 bags be sampled.

No. of bags in lot	No. of bags to sample
7	6
10	6
23	7
50	10
100	15
200	25
300	30
400	30

Sampling intensity for small containers

If the seed is in small containers such as tin, cartons or packets as used in retail trade, the following procedure is recommended by ISTA.

Basic unit	Seeds small containers
100 kg	20 containers, 5 kg each
100 kg	25 containers, 4 kg each
100 kg	100 cartons, 1 kg each

A 100 kg weight of seed is taken as the basic unit, and the small containers are combined to form sampling units not exceeding this weight (100 kg) e.g. 20 containers of 5 kg, 33 containers of 3 kg or 100 containers of 1 kg. For sampling purposes each unit is regarded as "one container" and the sampling intensity is as prescribed for seed in containers.

Sometimes it is said that if the bag or container size is less than 15 kg, a 100 kg weight of seed is taken as the basic unit and the small bags or containers are combined to form the sampling units not exceeding this weight (100 kg). But if the bag or container size is 15 kg to 100 kg then every bag or container will be considered as a basic unit and the sampling intensity will be as prescribed for seed in containers.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

Methods of obtaining working samples in the laboratory

The primary samples are drawn from a number of places of the seed lot with the help of trier or hand. The individual primary samples are mixed together to form composite sample. The composite sample is mixed thoroughly by hand and it is divided into two equal parts. One half is discarded and the second half is again mixed thoroughly and divided in the same way. The process of mixing and dividing is repeated until the desired size of the submitted sample is obtained.

Working samples can be obtained by two methods described in the International Rules of ISTA (1993).One is the submitted sample is divided using mechanical dividers and the other types where the division is done manually.

The minimum sizes of working samples for purity analysis prescribed under the International Rules of ISTA (1993) are calculated to contain at least 2500 seeds. Usually, the sample size of 400 seeds is used in different seed health tests, but for some seed-borne fungi a bigger number of seeds may be required e.g., *Phoma lingam* in seeds of *Brassica spp.*, loose smut infection in wheat and barley and downy mildew fungi in different crops.

Mechanical methods

The methods are suitable for all kinds of seeds except the extremely chaffy types. In principle the apparatus divides a sample into two approximately equal parts. The submitted sample can be mixed by passing it through the divider, recombining the two parts and passing the whole sample through a second time, and similarly, a third time, if necessary. The sample is reduced by passing the seed through repeatedly and removing one bald on each occasion. This process of successive halving is continued until a working sample of approximately, but not less than the required size is obtained. The dividers described below are example of suitable equipment. Specially two types of seed divider are used to divide the seed into two equal portions.

Conical divider

The conical divider, Boerner type is produced in two sizes- the smaller and the bigger one. The smaller is suitable for small-seeded species (less than the size of *Triticum* spp.) and the bigger for larger-seeded species (the size of *Triticum* or larger). However, in case of grasses the big divider is used. It consists of a hopper, cone and series of baffles directing the seed into two spouts. The baffles form alternate channels and spaces of equal width. They are arranged in a circle at the summit and are directed inwards and downwards, the channels leading to one spout and the spaces to a second spout. The flow of seed from the hopper is controlled by a valve. When the valve is opened, the seed falls by gravity over the cone where it is evenly distributed to the channels and spaces, then passes through the seed spouts into the seed collection containers. The width and number of the channels and spaces are important. For the larger divider, 19 channels and 19 spaces each 25.4 mm wide, and for the small divider 22 channels and 22 spaces each 7.9 mm wide have been found satisfactory.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

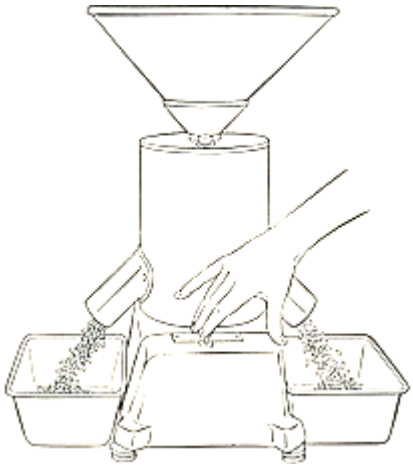


Figure. Seed divider

According to the International Rules of ISTA, for purity test the submitted and working seed sample sizes are as follows:

Seed	Submitted sample (gm)	Working sample(gm)
Rice	700	70
Wheat	1000	120
Maize	1000	900
Barley	1000	120
Pigeon pea	1000	300
(Thick pea	1000	1000
Black gram	1000	700
Lentil	600	60
Field pea	1000	900
Cowpea	1000	400
Soybean	1000	500
Groundnut	1000	1000
Mustard	40	4
Linseed	150	15
Cotton	1000	350
Jute	150	15
Shanpat	600	60
Chilli	150	15
Tobacco	25	0.50

Moisture test

The seed moisture content (mc) is the amount of water in the seed. It is usually expressed as a percentage on wet weight basis in any seed-testing laboratory. The seed moisture content is the most vital parameter, which influence the seed quality and storage life of the seed. Seed moisture content is closely associated with several aspects of physiological seed quality. For example, it is related to seed maturity, optimum harvest time, mechanical damage, economics of artificial seed drying, seed longevity and insect & pathogen infestation.

Definition

The moisture content of seed sample is the loss in weight when it is dried in accordance with 1STA rules. It is expressed as a percentage of the weight of the original sample.

Principle

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

The methods prescribed are designed to reduce oxidation, decomposition or the loss of other volatile substances while ensuring the removal of as much moisture as possible.

Seed ageing and loss of seed germination

Seed ageing and loss of germination cannot be stopped, but can be minimized by proper seed storage conditions. The important environmental factors influencing germination loss are relative humidity (RH) governs the seed moisture, and temperature, higher are these; the more rapid is the seed deterioration. The effects of seed moisture and temperatures are summarized succinctly in Harrington's rules, as under:

Determination of seed moisture content

As seed moisture and its management influences so many physiological seed quality parameters essential to seed quality. Seed moisture measurement appropriate to the purpose is needed in commerce and research. The optimum method for moisture testing depends upon:

1. Chemical composition of seed
2. Seed structure
3. Moisture content level
4. Degree of accuracy and precision required
5. Constraints of time
6. Technical expertise and cost

The ideal could be that is adopted to all seeds, measures moisture content from 0 to 100 percent, reproducible, require less training and low in cost. It is impossible to combine all these. However, in order to measure the moisture content of seeds, methods can be broadly grouped in two categories:

a) Direct method
b) Indirect method

Direct method

Under this category, the seed moisture content is measured directly by loss or gain in seed weight. These are:

1. Desiccation method
2. Phosphorus pentaoxide method
3. Oven-drying method
4. Vacuum drying method
5. Distillation method
6. Karl Fisher's method
7. Direct weighing balance
8. Microwave oven method

Indirect method

These are no so accurate; estimation is approximate, but convenient and quick in use. These are frequently used at seed processing plants. These measure other physical parameters like electrical conductivity or electrical resistance of the moisture present in the seed. Values are measured with the help of seed moisture meters, and these values are transformed into seed moisture content with the help of calibration charts, for each species, against standard air-oven method or basic reference method.

Above all Karl-Fisher's method has been considered as the most accurate and the basic reference method for standardizing other methods of seed moisture determination. The constant temperature oven drying method is the only practical method, approved by International Seed Testing Association (ISTA) and other organization to be used for routine seed moisture determination in a seed-testing laboratory.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

Constant temperature oven drying method

The constant temperature oven drying method is broadly grouped into two categories:

1) Low Constant Temperature Oven Method
2) High Constant Temperature Oven Method

Low constant temperature oven method: This method has been recommended for seed of the species rich in oil content or volatile substances (Table I). In this method, the pre- weighed moisture bottles along with seed material are placed in an oven maintaining a temperature of 103°C. Seeds are dried at this temperature for 17°±1 hr. The relative humidity of the ambient air in the laboratory must be less than 70 percent when the moisture determination is carried out.

High constant temperature oven method: The procedure is the same as above except that the oven is maintained at a temperature of 130°-133°C. The sample is dried to a period of four hours for *Zea mays*, two hours for other cereals and one hour for other species. In this method there is no special requirement pertaining to the relative humidity of the ambient air in the laboratory during moisture determination.

Essential equipments and supplies

1) Constant temperature precision hot-air electric oven.
2) Weighing bottles/Moisture containers.
3) Desiccator with silica gel.
4) Analytical balance capable of weighing up to 1 mg.
5) Seed grinder/An adjustable grinding mill.
6) Tong.
7) Heat resistant gloves.
8) A brush/A steel brush.

Period of seed drying

The prescribed period of seed drying shall be 17°± 1 hrs at 103°C under low constant and 1 to 4 hrs at 130°-133°C under high constant temperatures. Maize seed be dried for 4 hrs, cereals and/or other millets for 2 hrs and the remaining species for 1 hr. Seeds rich in oil content or with volatile substances be dried for 17°±1 hrs under low constant temperature.. See drying period begins from the time oven returns to maintain the desired temperatures.

Sample size

The ISTA rules recommend that two replicates, each with 4 gm of seed be used for determination of seed moisture content. This seed sample weight may be modified to 0.2 to 0.5 gm per replicate, with precise weighing, for use in seed genebanks, to avoid unnecessary depletion of precious biological resources.

Procedure

- Seed moisture determination be carried out in duplicate on two independently drawn working samples.
- Weigh each bottle with an accuracy of 1 mg or 0.1 mg.
- First weigh the empty bottle/container with its cover.
- Grind the seed material, evenly using any grinder/grinding mill that does not cause heating and/or loss of moisture content.
- Mix thoroughly the submitted sample, using spoon, and transfer small portions (4 to 5 gm) of seed samples directly into weighing bottles/containers, by even distribution on bottom of the containers.
- After weighing, remove the cover or lid of the weighing bottles/containers.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

- Place the weighing bottles/containers in an oven, already heated to or maintaining the desired temperature, for the recommended period.
- At the end of seed drying period, weighing bottles/containers be closed with its lid/ cover.
- Transfer the weighing bottles/containers to the desiccators having silica gel (self indicating - blue), to cool down for 40 to 45 min.
- Weigh again the cooled weighing bottles/containers.
- Calculate the seed moisture content.

Calculation of results

The moisture content as a percentage by weight (fresh weight basis) is calculated to one decimal place, by using of the formulae:

$$\% \text{ seed moisture content (mc)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where

M_1 = Weight of the weighing bottle/container with cover in gm
M_2 = Weight of the weighing bottle/container with cover and seeds before drying
M_3 = Weight of the weighing bottle/container with cover and seeds after drying

{Note: The seed moisture determination must be done in two replicates, with precise weighing (i.e. up to three decimal places) using lightweight weighing bottles/containers.}

Use of tolerances

Result is the arithmetic mean of the duplicate determination of seed moisture content, for a given seed sample. The maximal difference of 0.2% is recommended between two replicates, for crop seed species under ISTA rules. If the difference between two replicates exceeds 0.2%, the seed moisture determination in duplicate be repeated.

As it is very difficult, rather impossible to, meet the replicate difference of seed moisture up to 0.2% in tree or shrub species, maximal limit of 0.3 to 2.5% is recommended between two replicates for seed moisture in tree or shrub species under ISTA rules.

Reporting of results

Seed moisture content be reported to the nearest 0.1% on ISTA analysis certificate.

If the seed moisture content is determined using any moisture meter, the brand name and type of the equipment be mention on the analysis certificate, under column of "other determinations" reporting of range for which the moisture meter is calibrated is the another requirement, on seed analysis certificate.

Species for which the low constant temperature (103°C) oven method:

Allium spp
Linum ustatissimum
Arachis hypogea
Raphanus sativus
Brassica spp.
Rlcnus communis
Sesamum indicum
Capsicum spp.
Sesamum orientale
Glycine max
Sinapsis spp.
Gossypium spp.
Solanum melongene

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman

Species for which high constant temperature (130 to 133°C) oven method:

Agrostis spp.
Citrullus lanatus
Lolium spp.
Phaseolus spp.
Cucumis spp.
Lotus spp.
Cucurbita spp.
Lupinus spp.
Pisum sativum

Purity

Concept

In common terms purity is an expression of how 'clean' the seed lot is.

Purity of a seed lot indicates in percentage how large a fraction is made up of pure seeds of the species in question, and how much is made up of inert matter and other seeds. Impurities may be any non-seed material (leaf, flower, fruit fractions, soil etc.), small fractions of seeds of the actual species, as well as seeds of other species.

Purity is expressed as the weight percentage of pure seed fraction over the total weight of the working sample:

$$\text{Purity} = \frac{\text{Weight of pure seed}}{\text{Total weight of working sample}} \times 100$$

Components of seed purity and their characteristics

There are 3 major components of a seeds lot carried out for purity analysis. These are:

1. Pure seed
2. Other crop seed
3. Inert matters

1. Pure seed

The pure seed shall refer to the species stated by the sender, or found to Predominate the test, and shall include all botanical varieties and cultivars of that species (even if immature, undersized shriveled disease or germinated providing. They can be definitely identified as of that species) unless transformed into visible fungal-sclerostia, smut balls or nematode galls. Pure seed shall include a) intact seed units (commonly found as dispersal units i.e. achenes and similar fruits, schizocarp, florets etc) as defined for each genus or species; b)pieces of seed units larger than, one half their original size.

Seed units of families Fabaceae (Leguminaceae), Brassicaceae (Cruciferae), Cupressaceae, Pinaceae and Taxodiaceae with the seed coat entirely removed shall be regarded as inert matter. Separated cotyledons of Fabaceae are regarded as inert matter, irrespective of whether or not the radicle plumule axis and/or more than half of the tests may be attached.

Definition of Pure Seed for specific crops

Poaceae

Oryza spp. (Paddy)

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman

- Spikelet, with glumes, lemma and palea enclosing a caryopsis including the awn irrespective of its size.
- Floret, with or without lemmas, with lemma and palea enclosing a caryopsis including the awn irrespective of its size.
- Caryopsis.
- Piece of caryopsis larger than one-half the original size.

Triticum, *Zea*, *Secale* (Wheat, Maize, Triticale)

- Caryopsis
- Piece of caryopsis larger than one-half the original size.

Hordeum spp. (Barley)

- Floret, with lemma and palea enclosing a caryopsis with or without awn or with or without rachis segment irrespective of their length.
- Piece of floret containing a caryopsis larger than one-half the original size.
- Caryopsis
  Piece of caryopsis larger than one-half the original size.

Avena (Oat)

- Spikelet with lemma and palea enclosing a caryopsis, with or without awn plus attached sterile floret.
- Floret with lemma and palea enclosing a caryopsis, with or without awn.
- Caryopsis
- Piece of caryopsis larger than one-half the original size.

Sorghum

- Spikelet, with glumes, lemma and palea enclosing a caryopsis with or without hyaline palea or lemmas, rachis segments padicle(s),awn(s),attached sterile or fertile floret(s).
- Floret, with lemma and palea ,with or without awn.
- Caryopsis.
- Piece of caryopsis larger than one-half the original size.

Fabaceae (Gram, Pea, Mungbean, Soybean, Sunhemp)

- Piece of seed larger than one-half the original size with testa.
- Seeds and pieces of seed without testa is regarded as inert matter. Separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis or more than half of the testa may be attached.

Brassicaceae (Radish, Mustard, Cabbage and Cauliflower)

- Piece of seed larger than one-half the original size with testa.
- Seeds and pieces of seed without testa is regarded as inert matter. Separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis or more than half of the testa may be attached.

Solanaceae (Chillies, Brinjal, Tomato, Tobacco)

- Seed with or without testa.
- Piece of seed larger than one-half the original size with or without testa.

Liliaceae (Onion, Garlic)

- Seed with or without testa.
- Piece of seed larger than one-half the original size with or without testa.

Amaranthaceae (*Arnaranthus*)

- Seed with or without testa.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

- Piece of seed larger than one-half the original size with or without testa.

Cucurbitaceae (Watermelon, Muskmelon, Cucumber, Pumpkin, Squash, Bottle guard)

- Seed with or without testa.
- Piece of seed larger than one-half the original size with or without testa.

Malvaceae (Cotton)

- Seed with or without testa (testa with or without fuzz).
- Piece of seed larger than one-half the original size with or without testa.

Compositeae (Sunflower, lettuce, chicory)

- Achene, with or without beak, or with or without pappus, unless it is obvious that no seed is present.
- Piece of achene larger than one-half the original size, unless it is obvious that no seed is present.
- Seed with the pericarp/testa partially or entirely removed.
- Piece of seed larger than one-half the original size, with the pericarp/testa partially or entirely removed.

2. Other Seed

a. Other crop seed: Other crop seed shall include seed units of any plant species other than of pure seed grown as crops.

b. Weed Seed: Seeds, florets, bulblets, tubers, or sporocarps of plants recognized as weeds by laws, regulation or by general usage shall be considered weed seeds. Further classification of species is determined with the use of the reference, “Uniform Classification of Weed and Crop Seed” which is published by the Association of Official Seed Analysts.

3. Inert Matter

Soil particles, stones, chaff, stems, leaves, flowers, cone scales, pieces of bark, pieces or resin, etc.. Pieces of broken and damaged seed units of crops which are half the original size or less. Damaged weed seed with over half the embryo missing.

Inert matter includes seed units and all other matter and structures that are not defined as pure seed, other crop seed or seed as follows:

Seed units in which it is readily apparent that no true seed is apparent.

- Pieces of broken or damaged seed units half or less than half the original size.
- Those appendages not classed as being part of pure seed in the pure seed definitions must be removed and included in the inert matter.
- Seed of Fabaceae, Brassicaceae, Cupressaceae, Pinaceae and Taxodiaceae with the seed coat entirely removed.
- Unattached sterile florets, empty glumes, lemmas, paleas, chaff, stems, leaves, cone scales, wings, bark, flowers, nematode galls, fungus bodies such as ergot, sclerotia and smut balls, soil, sand, stones and all other non seed matter.

Each of the four component parts is weighed and a percentage is calculated from the sum of the four component parts. This purity information is used to tag the seed for sale and is reported on the analyst’s Report of Analysis.

Purity Test

Equipments

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

Aids such as transmitted light, sieves and blowers may be used in separating the component parts of the working sample. The blower to be used for the uniform blowing method for species of family Poaceae.

Other equipments required are:

- Dividers
- Balance
- Blowers
- Diaphnoscope using reflected light are used to separate inert matter such as empty florets of grasses.
- Sieves.
- Sample pans, dishes, forceps, spatula and hand lens
- Seed herbarium of crop and ,weed seed

Procedure

1. The submitted sample was reduced in size by a gamete seed divider to sufficient size for analysis. This size is usually based on appropriate weight of 2500 seeds and is referred to as the working sample.

2. The working sample was weighted and recorded the exact weight in gram to the minimum of decimal places necessary to calculate the percentage of its components to one decimal place is indicated below.
3.

Wt. of working sample (g)	No. of decimal place
Less than 1.0000	4
1.0000 to 9.999	3
10.000 to 99.99	2
100.00 to 999.9	1
1000	0

4. The working sample was placed on the working board/ white offset paper. Then sample was separated into four component, pure seed, other crop seed, weed seed and inert matter.
5. The individual components were weighted to the appropriate number of decimal places.

Experimental Data:

Weight of total working sample (W) =
Weight of pure seed (W_1) =
Weight of other seed (W_2) =
Weight of inert matter (W_3) =
Weight of all components (W_4) = $W_1 + W_2 + W_3$

6. Calculation of the results were done in the following way-

% of the pure seed $= \frac{W_1}{W_4} \times 100$ =

% of other seed $= \frac{W_2}{W_4} \times 100$ =

% of inert matter $= \frac{W_4}{W_4} \times 100$ =

7. The results of purity analysis are given to one decimal place and the percentage of all components must be rounded. Components of less than 0.05% shall be reported as trace.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

Percentage of any crop seed from other crop seed components is found 5% or more will be considered as second pure seed.

Precautions:

1. Weight of components should be taken rapidly without loss of time to overcome variation of weight that may be due to moisture component change in sample.
2. Consideration of decimal places should be followed according to the standard rule thus care should be taken during weighing of working sample and, different components.
3. The sample should not be less than 0.5 g and more than 1000 g.

Rounding procedure

Add together the percentages of all fractions. Fractions that are to be reported as a "trace" are excluded from this calculation; the other fractions shall then together total 100.0%. If the sum does not equal 100.0% (either 99.9 or 100.1) then add or subtract 0.1% from the largest value (normally the pure seed fraction).

Note: If a correction of more than 0.1% is necessary, check for a calculation error.

Germination test

Seed germination tests measure the number of healthy well-developed seedling under laboratory conditions, not just whether a root has emerged from the seed. Because of this a germination test will take at least 7 days for cereals or for grasses it can take 4 weeks.

The process of seed germination is complex and can be affected at different stages by many factors and interactions of factors such as temperature, water availability, oxygen, light, substrate, maturity of seed, physiological age of seed. In laboratory germination tests these factors are optimised in order to measure the maximum number of seeds capable of producing healthy well-developed seedlings. A laboratory germination test does not take into account the effects of non-optimal conditions on the seed. It is therefore useful to view a laboratory germination test result as potential rather than absolute emergence.

Percentage of germination

The percentage of germination indicates the proportion by number of seeds which have produced normal seedlings within specified period under favorable conditions. It is calculated by using the following formula-

$$\text{Percentage of germination} = \frac{\text{No. of normal seedlings}}{\text{No. of seeds set for the test}} \times 100$$

Substrata/media used: Filter paper, sand, soil, saw dust etc.

Methods of testing for laboratory germination of crops seeds

Crop	Substrate	Temp °C	First count (days)	Second count	Additional
Maize *Zea mays*	BP,S	20-30 25,20	4	7	KNO_3
Sorghum *Sorghum bicolour*	TP,BP	20-30 25	4	10	Prechill

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman

Crop	Substrate	Temp °C	First count (days)	Second count	Additional
Beans *Phaseolus spp*	BP,S	20-30 25,20	5	9	
Pearl millet *Pennisetum glaucum*	BP,TP	20-30 20-35	3	7	
Rice *Oryza sativa*	TP,BP,S	20-30 25	5	14	Preheat(50C);Soak in H_2O or HNO_3(24
Wheat *Triticum aestivum*	TP,BP,S	20	4	8	Preheat(30-35C) Prechill: GA
Cowpeas *Vigna unguiculata*	BP,S	20-30 25	5	8	
Lentils *Lens culinaris*	BP,S	20	5	10	Prechill
Chickpea *Cicer arietinum*	BP,S	20-30 20	5	8	
Groundnuts *Arachis hypogaea*	BP,S	20-30 25	5	10	Remove shells,Preheat(40C)

Notes:

1. Substrate: BP=Between paper, TP=top of paper, S=in sand,
2. Temperature: single numbers indicate constant temperature. Two numbers indicate alternating temperatures with 16 hours at the first temperature and 8 hours at the second temperatures.
3. Light should be provided by a cool white fluorescent source of 750-1250 lux. The seed should be illuminated for at least 8 hours in every 24 hour period.

Source: ISTA Rules for Seed Testing.

Evaluation of seedlings

A. Normal seedling

1. A well developed root-system including a primary root except for those plant normally producing seminal roots.
2. A well developed and intact hypocotyle and epicotyle without damage to the tissues and normal plumule.
3. In the poaceae (gramineae), a well developed primary leaf within or emerging through the coleoptile.
4. One cotyledon for seedlings of monocots and two cotyledons for seedlings of dicot.

B. Abnormal seedling

1. Seedling which was lacking in any one of its essential structures or the structures grown were not balanced.
2. All damaged, deformed and decayed seedlings.
3. Seedlings short and weak or spindly or watery.
4. Frost-damaged seedling with grainy coleoptile or plumule and primary leaves which are

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

weak and spirally twisted.
5. Seedling which fails to develop a green color.

C. Hard seed

Seeds of Fabaceae (Leguminosae) and Malvaceae, which remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seed coat, are classified as hard seeds.

D. Dead seed

Seeds which at the end of the test period are neither hard nor fresh and have not produced seedlings are classified as dead seed.

Viability Test

Viability of seeds indicates that a seed contains structures and substances including enzymatic systems which give it the capacity to germinate and produce normal seedlings under favorable conditions in the absence of dormancy. Such a viable seed may or may not be readily or immediately germinable. Dormant viable seeds may require lengthy specific treatments before they become immediately germinable.

Only two methods, the topographical tetrazolium test and the embryo excision test were previously accepted by the International Seed Testing Association as official methods for some species of seeds. ISTA has recently accepted the X-ray method as a valid alternative to the cutting test for the detection of empty and insect-damaged seeds. The following tests can be applied depending on the circumstances:

Cutting test

The simplest viability testing method is direct eye inspection of seeds which have been cut open with a knife or scalpel. If the endosperm is of normal colour with a well developed embryo, the seed has a good chance of germinating. This test is not very reliable. Seeds with milky, unfirm, mouldy, decayed, shrivelled or rancid-smelling embryos and abortive seeds that have no embryo can be judged as non-viable without much difficulty (Bonner 1974). But it is not possible to distinguish moribund, recently dead or recently injured seeds which still appear the same as sound ones. The cutting test, as already mentioned, is used at the end of a germination test to determine the apparent viability of ungerminated seeds; it is also a useful tool in estimating the size and maturity of the seed crop before collection (Chapter 3) and the efficiency of methods used in processing.

Topographical tetrazolium test

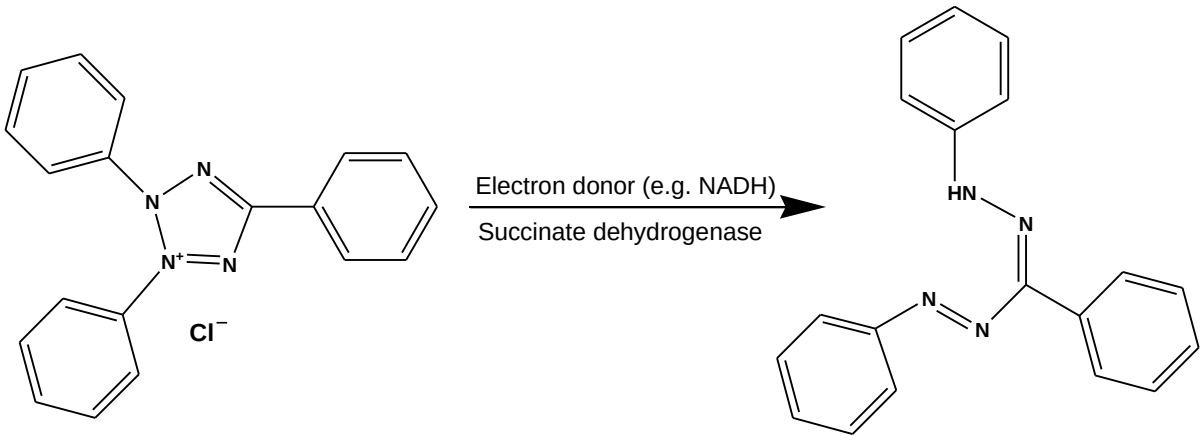
Topographical tetrazolium or TZ test is very useful for rapidly obtaining an indication of germination potential and viability of samples and is in extensive use.

Principles

In this biochemical test, living cells are made visible by reduction of an indicator dye. The indicator used in the TZ test is a colorless solution of a tetrazolium salt imbibed by the seed. Within the seed tissues, it interferes with the reduction processes of living cells and accepts hydrogen from the hydrogenases. By hydrogenation of the 2,3,5-triphenyl tetrazoliurn chloride, a red, stable and non-diffusible substance, triphenyl formazan is produced in living cells. The reaction is as follows:

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com



2,3,5 triphenyl tetrazolium chloride (white) 2,3,5 triphenyl formazan (red)

This makes it possible to distinguish the red color living parts of seeds frown the colorless dead ones.

Field of application

The test is not valid for previously germinated seeds and must not be applied to submitted samples, which contain any dry germinated seed.

Method of Tetrazolium Testing

A. Testing sample
A representative sample of fifty or one hundred seeds is usually sufficient for most practical tetrazolium tests.

B. Equipments

- Petridish
- Cutting, piercing, and cracking devices
- Forceps/tweezers
- Magnifying device
- Dropper
- Brown colored bottle
- Needles
- Conditioning (seed moistening) media
- Oven or incubator

Preparation of tetrazolium solution

Several concentrations of tetrazolium solution may he used with comparable results. The 1% solution is used for seeds that are not bisected through the embryo. while the (0.1% solution is used for seeds in which the embryo is bisected. Other low concentrations such as 0.2% and 0.5% are sometimes used instead (1.0 or 0.1.1% solution). The p^H of the solution should be between six and eight for best staining to occur if the pH of the water is not in the natural range. The tetrazolium salt should be dissolved in a phosphate buffer solution. The buffer solution is prepared as follows:

Solution I: Dissolve 9.078 g of KH_2PO_4 in 1 litre of water.

Solution 2: Dissolve 11.876 g of $Na_2HPO_4.H_2O$ in 1 litre of water.

Take 400 ml of solution I and 600 ml of solution 2 and mix them together. In a liter of buffer solution prepared as above. dissolve 5 g of tetrazolium salt. This gives 0.5% tetrazolium solution of pH 7.0.

The solution should be stored in brown bottle to prevent photoreaction.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

D. Preparation for tetrazolium Test

a. Seeds of gram may swell so rapidly and irregularly when placed directly in water or tetrazolium solution that frequently the seed coat burst. Cotyledons separate. Hypocotyls break, or other damage occurs. It is preferable to condition these seeds slowly in moist paper towels overnight before staining. So that they absorb moisture without damage to the seed. Staining time may be reduced by puncturing or cutting the seed coats.

b. Staining: The prepared seed should be placed in suitable container (petridish) and and covered with the testing solution (about 80 ml, and keeping it in a dark warm place (40°C) for 2-8 hours.

c. Evaluation of samples

The sample is ready for evaluation when it is stained. The evaluation method for gram seed is as below:

Magnification of 7 X desirable for the small-seeded species but not necessary for larger seeds.

Germinable seeds:

1. The embryo is well-developed, non-fractured. and of a normal red color and condition
2. The embryo contains no more than the maximum listed for one or more of the following:
   i. Small, shallow, unstained or intensively stained areas on outer solaces of cotyledons.
   ii. One cotyledon completely fractured at point of attachment, or complete transverse fracturing of both cotyledons with no more than one-half of the cotyledon tissue non-functional.
   iii. Unstained areas near the embryonic axis attachment on either cotyledon, which does not involve vascular tissues of at least one cotyledon.
   iv. Shallow non-stained areas on hypocotvls.

Non-germinable seeds:

1. The complete embryo or a major portion of it is not stained and is of dull appearance and flaccid, or is of distinctly abnormal colour or texture.
2. Embryo with deep-seated deterioration of cotyledon tissues when slight pressure is applied.
3. Embryo with deep-seated deterioration of cotyledon tissues that extends to inner flat surfaces.
4. Embryo with both cotyledons functionally severed from embryonic axis by fractures or deteriorated tissues, or by transverse fractures or deteriorated areas that cause more than one-half of the total cotyledon tissues to be non-functional.
5. Embryo with extensive surlitce necroses involving vascular tissues, or extensive mottling of brownish or bluish red and white staining patterns.
6. Embryo with deteriorated areas on hypocotyls that involve more than one-half of the diameter of the stele.
7. Embryo with deterioration of radicle that extends, upward and beyond the tapering. or angular cell division area, of the stele.
8. Embryo with epicotyl or both plunndes made non-functional by fractures.
9. Embryo with necroses of plumules. especially frequent in snap beans, that cause more than one-half of the plumule surfaces to the non functional. In borderline cases. the pair of plumules should be broken loose for observation on both sides.

Excised embryo test

By this method, the seeds are soaked for 1 – 4 days and the embryos are then excised from the seeds and placed on moist filter paper or blotter discs in petri dishes. The tests are placed in the light at a constant temperature of 20°C. The condition of the embryos is examined daily. Depending upon the species and lot differences, the tests can be terminated after only a few days, up to a maximum of 14 days, or as soon as distinct differentiation into viable and non-viable embryos can be made.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

The excised embryo test is similar to germination tests in that it measures the quality of the seed by their actual germination. In addition it allows some measure of the embryo dormancy to be made, by counting those seeds which, although not growing normally, have grown slightly, remained firm and have kept their colour for the test period. The test is not valid for previously germinated seeds and must not be applied to samples which contain any dry germinated seeds. The success of the test requires considerable skill and experience in the operator and the ISTA rules restrict it to only a few species.

In a comprehensive study, Schubert (1965) compared the excised embryo method with the tetrazolium method for determining the viability of dormant tree seeds. He concluded that the tetrazolium method should receive preference over the embryo excision method but that improvements in the tetrazolium test should be made by providing for the use of bactericides and stronger reducing solutions to resolve doubts in weakly stained tissues.

Radiographic methods

Radiography was first used to determine seed quality over 70 years ago (Lundstrom, 1903, cited by Kamra 1964). The studies of Simak and Gustafsson (1953) highlighted the X-ray technique as a diagnostic method of tree seed analysis. The X-ray contrast method which uses various contrast or radiopaque agents was developed and applied successfully to species of *Pinus* and *Picea* (Simak 1957; Kamra 1963 a, 1963 b).

The X-ray method permits the detection of empty seeds, mechanical damage and abnormally developed internal seed structures, measurement of the thickness of the seedcoat and assessment of the seed viability when combined with a contrast agent.

The X-ray contrast method is based on the principle of semipermeability. When seeds are treated with a contrast agent, for example aqueous $BaCl_2$ or vaporous $CHC1_3$, their living tissues are able to prevent its entry due to their semi-permeability, but the dead tissues become impregnated. The impregnated tissues absorb X-radiation more intensively than the unimpregnated ones and thus appear lighter on the film than the unimpregnated ones. The contrast permits living and dead tissue to be located in the seed and an estimation of its viability (Kamra 1964). There are now possibilities of using non-toxic water, instead of toxic $BaCl_2$ or $CHC1_3$, as a contrast agent for testing seed viability (Simak 1982).

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) has a stimulating effect on seed germination and has been used in a rapid test for germination of several conifers in the western USA (Bonner 1974). Seeds are soaked overnight in 1% H_2O_2. The seedcoat is then cut open to expose the radicle tip and the seeds put back into 1% H_2O_2 in the dark at alternating temperatures (20° and 30°C). Counting and refreshment of H_2O_2 is done after 3 or 4 days and final assessment after 7 or 8 days. Radicle growth of 5 mm or more is scored “evident”, 0 – 5 mm “slight” and no growth means a nonviable or empty seed (Danielson 1972 cited by Bonner 1974). The test is quicker but less reliable than a normal germination test (usually producing a more rapid and higher final germination), slower but simpler to perform than excised embryo and easier to interpret than TZ.

Rapid tetrazolium seed viability

Test description: Tetrazolium test, based on international protocols. Seeds are pre-moistened, embryos removed, stained in tetrazolium and visually assessed. Chemical damage, dormancy, seedling disease are not detected. Detailed assessments of damage are not included in the test.
Result reported: Results reported as % viable seeds. Major quality problems observed on a sample, may be noted but not quantified on the test report.

Advantages: Rapid indication of seed viability. Can assess potential germination quality in the presence of dormancy.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

Seed vigour tests

Seed vigour can be considered the closest measure of potential field performance. For seedlings classified as normal by a germination test, there will in fact be differences between the seedlings in their ability to perform well under a wide range of environmental conditions. Vigour tests aim to measure the ability of the seed to perform well under unfavourable conditions and are used for two main reasons:

- To discriminate between seed lots for suitability for storage.
- To discriminate between seed lots for planting value in relation to optimising establishment (e.g. to promote synchronous emergence or maximal performance under sub-optimal seedbed conditions).

Vigour tests are often species specific and so a large number of tests are in existence. It is important to chose the most appropriate test available and understand its limitations. Tetrazolium vigour tests for example, measure vigour indirectly, and therefore treat vigour as an intrinsic property of the seed. Some, such as physiological stress tests, measure susceptibility to unfavourable conditions directly.

Rapid tetrazolium seed viability & vigour (most species)

Test description: Tetrazolium test, based on international protocol. Seeds are pre-moistened, embryos removed, stained in tetrazolium and visually assessed into vigour categories. Chemical damage, dormancy, seedling disease are not detected. Detailed assessments of damage are not included in the test.
Result reported: Results reported as % viable seeds, with sub-categories for % high vigour seed, % medium vigour seed and % low vigour seed. Major quality problems observed on a sample, may be noted but not quantified on the test report.
Advantages: Rapid indication of seed viability and potential vigour. Can be useful where seedbed conditions are unlikely to be ideal or where there is a choice of lots to be used for seed or storage. Can assess potential germination quality in the presence of dormancy.

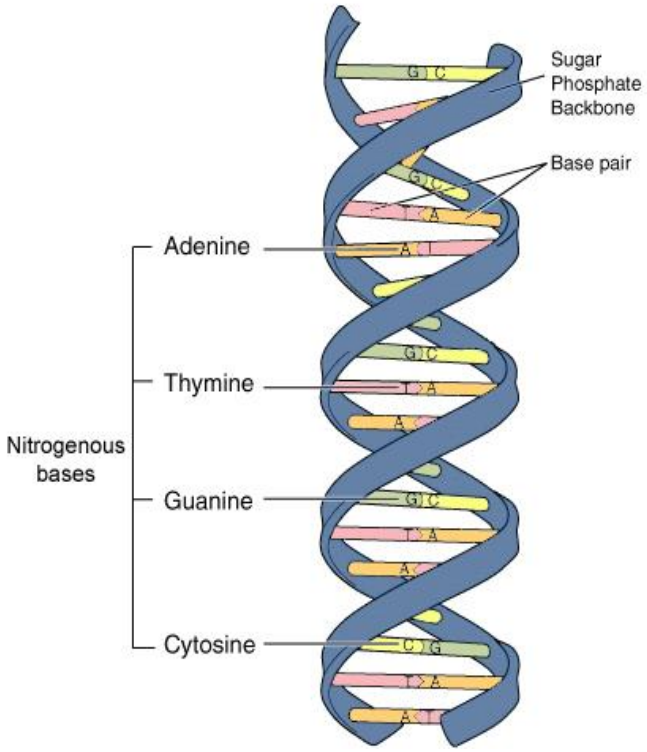
Common problems with seed quality

- Wheat tends to show good germination. Seedling diseases can affect germination test performance, especially in wet years.
- Barley seed tends to show good germination. Seedling diseases can affect germination test performance, especially in wet years. Dormancy in early season seed can make germination assessment more difficult. Mechanical damage can be problematic, especially in dry years. The embryo is exposed and so is more vulnerable to damage.
- Field bean seed quality is usually very variable due mainly to problems with seed being mechanically damaged. The problem is often worse in dry years.
- Oilseed rape quality can be problematic for a number of reasons, including heat damage, mechanical damage, chemical damage and occasionally disease.
- Heat damage can be a problem in any type of seed, due to artificial drying or excessively hot weather around harvest.
- The germination quality of over-yeared seed of any species is likely to have deteriorated, especially if storage conditions were not ideal. For over-yeared seed germination should always be re-checked prior to use.
- Chemical damage can be a problem in a number of situations. For example, if seed is stored in facilities used previously for potato storage. Residues of potato sprouting suppressant have the potential to seriously affect seed germination. Or if a crop has been accidentally sprayed with glyphosate the seed may not be killed but it can affect subsequent seed germination, especially in oilseed rape. For both these examples the seeds may be alive but will germinate abnormally and are less likely to produce healthy plants in the field, especially where conditions are less than optimum. Germination tests can identify chemical damage but tetrazolium tests cannot and would over estimate potential field emergence.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

DNA fingerprinting and barcoding for seed quality testing



- Each rung is made up of two bases that link together. There are four bases - adenine (A), thymine (T), guanine (G) and cytosine (C).
- Because of their chemical nature, A will only link with T and G will only link with C. No base can join with itself (i.e. NO A-A /T-T /G-G / C-C base-pairs).
- DNA has two strands. And the sequence of one strand determines the sequence of the other. If the base sequence on one strand of DNA is: GATCCTCATA

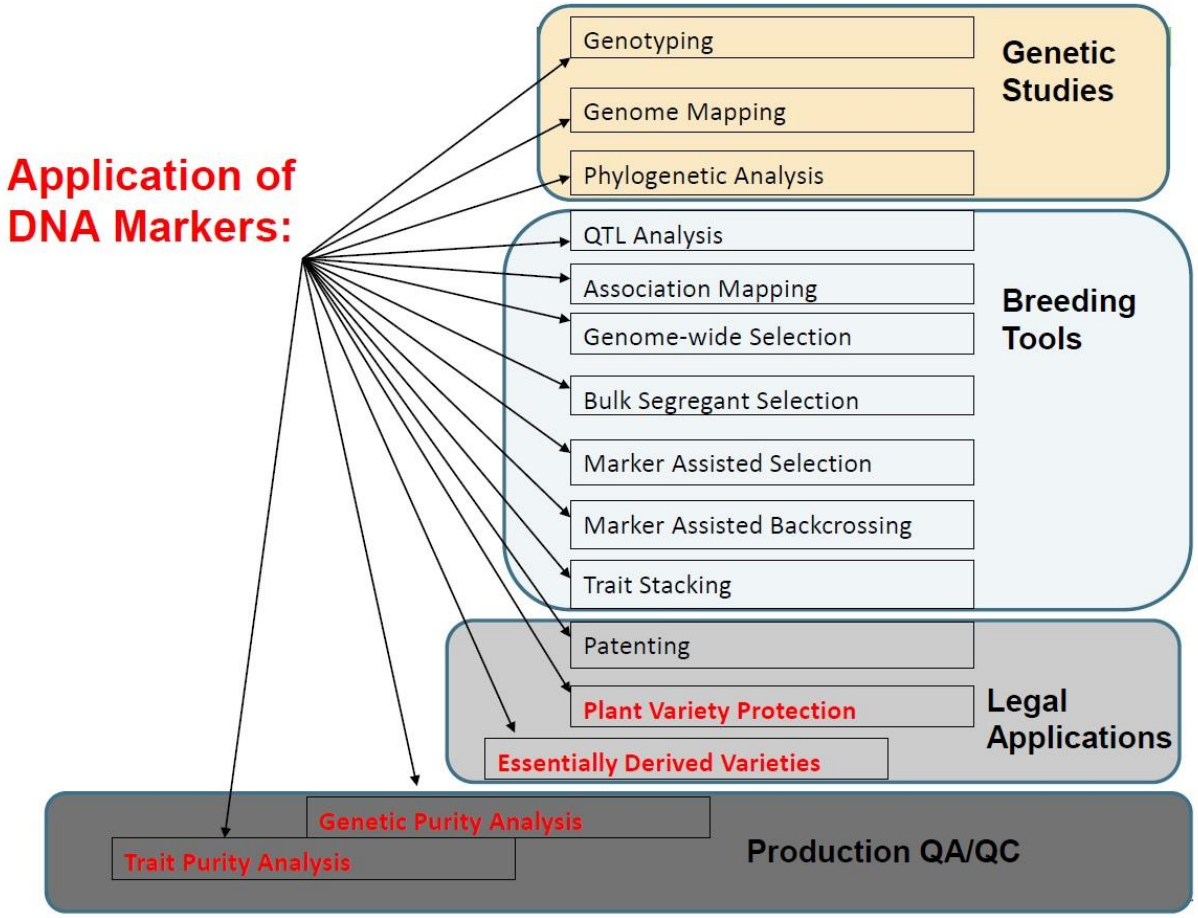
It is the story of 125 years back.

- Jeffreys AJ, Wilson V, and Thein SL (1885) Individual-specific 'fingerprints' of human DNA. Nature 316(6023):76–79
- Galton F (1892) Finger prints. Macmillan and Co., New York

Fingerprints were first accepted as evidence in court in 1905, DNA fingerprints were accepted by the judicial system within a couple of years after first publication of the technique. Since then, DNA fingerprinting has become a universal tool for the characterization of genetic differences and relatedness of individuals even thousands of years after their death

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com



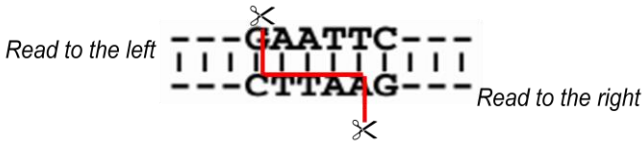
Using DNA Markers for Genetic Purity Testing

Based on DNA fingerprint, that is, genotyping N loci/markers Requirements:

- Polymorphic, have more then one allele represented in the population.
- ~Even Allele frequency
- No null alleles (where applies)
- Diagnostic
- Minimal number of markers (cost, efficiency)
- Robust performance

Restriction Enzymes

- A common use of restriction enzymes is to generate a "fingerprint" of a particular DNA molecule.
- DNA may be cut by some special naturally-occurring proteins called restriction enzymes.
- Each restriction enzyme only identifies and "cuts" at a very specific sequence (recognition site) in the DNA strand.
- Restriction enzymes typically recognise a symmetrical sequence of DNA, such as the site GAATTC.

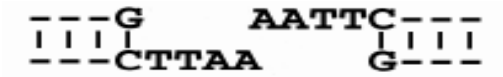


- The top strand is the same as the bottom strand when you read backwards.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman

- When the enzyme *EcoRI* cuts the strand between G and A, it leaves overhanging chains:



- These are termed "sticky ends" because the base pairs formed between the two overhanging portions will glue the two pieces together, even though the backbone is cut.

Electrophoresis

- DNA fragments of different sizes can be separated by using gel electrophoresis.
- DNA fragments carry negative charges. When there is a current flow, DNA fragments move towards the anode.

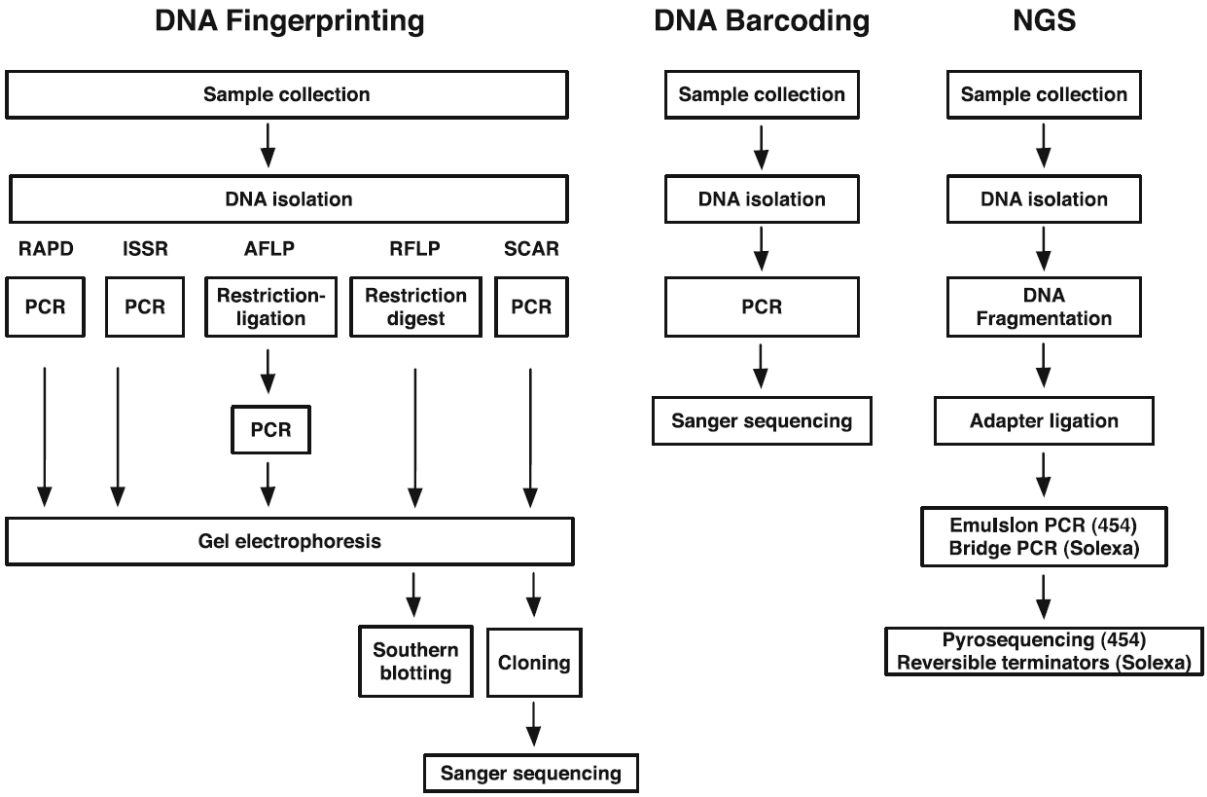


Fig. 1. Schematic comparison of the major steps involved in DNA fingerprinting, DNA barcoding, and next generation sequencing. *RAPD* random amplified polymorphic DNA, *ISSR* inter simple sequence repeat, *AFLP* amplified fragment length polymorphism, *RFLP* restriction fragment length polymorphism, *SCAR* sequence characterized amplified region, DNA barcoding, *NGS* next generation sequencing, *PCR* polymerase chain reaction.

Molecular marker fingerprinting

For the molecular fingerprinting seeds of the OPVs in question are obtained from the seed source, and 50–100 seeds are planted in the field or greenhouse. When the seedlings are about 2–4 weeks old, one leaf from at least 30 different plants are harvested. DNA is extracted from each plant, and equal amounts of the DNA from 15 plants are combined into a bulk. The extracted DNA is checked for quality and quantity, and the required working concentration prepared for polymerase chain reaction (PCR). PCR analysis of the two bulks is

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

performed using microsatellite markers (also called simple sequence repeats, or SSRs) that have been optimized for genotyping bulk samples.

DNA fingerprinting is a specialized analysis that is run using expensive laboratory equipment, reagents and software written specifically for this purpose so it can only be done by specialists, unlike the DUS study that can be run by anyone trained in cultivation and careful observation.

What are the requirements for DNA fingerprinting?

1. How should you sample a seed lot?

From each seed lot, obtain a representative sample, depending on the size of the seed lot, following the sampling method outlined by the National Seed Authorities (NSA). Official samples are submitted by the National Seed Authorities (NSA), but samples will be tested that were submitted by anyone. Tests will only reflect the sample that was delivered to the laboratory. Therefore, when submitting a sample, ensure that the seeds sent are a pure and known source of the seeds in question.

2. What seed sample size is required?

A seed sample of about 250 grams (or 100–200 seeds, for maize) can be sent to the laboratory for analysis. Up to 1000 grams should be collected at the time the sample is taken from the seed lot, and the remainder of the seed can be kept under cold storage until the tests are complete. If there are doubts regarding the results of the tests, a second sample can be drawn and sent to the laboratory again for testing.

3. What information is required before sending a seed sample?

Seeds should be submitted with the following information (an example is shown below):

1. Owner-
2. Address (postal)-
3. Crop- maize
4. Cultivar/Variety-
5. Lot No. (information from the owner/sellers, so that she/he can find the same lot or bag when the they get the results)
6. Size of sample- 100–200 seeds or 250 grams
7. Date sent-
8. Certified seed No/Yes
9. Tests required- DNA fingerprinting, germination

Challenges in the DNA Barcoding of Plant Material

DNA barcoding, using a short gene sequence from a standardized region of the genome, is a species identification tool which would not only aid species discovery but would also have applications ranging from large-scale biodiversity surveys through to identification of a single fragment of material in forensic contexts. To fulfill this vision a universal, relatively cheap, scalable system needs to be in place. The mitochondrial locus being used for many animal groups and algae is not suitable for use in land plants, and an appropriate alternative is needed.

This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

Progress has been made in the selection of two alternative regions for plant DNA barcoding. There are however many challenges in finding a solution that fulfils all the requirements of a successful, universally applicable barcode, and in the short term a pragmatic solution that achieves as much as possible and has payoffs in most areas has been chosen. Research continues in areas ranging from the technicalities of sequencing the regions to data analysis and the potential improvements that may result from the developing technology and data analysis systems.

The ultimate success of DNA barcoding as a plant identification tool for all occasions depends on the building of a reference database and it fulfilling the requirements of potential users such that they are able to achieve valid results through its use, that would be more time consuming and costly, and less reliable using other techniques.

In 2004, the Consortium for the Barcode of Life (CBOL) was founded, with funding from the Alfred P. Sloan Foundation, as an international initiative to promote and develop DNA barcoding (http://www.barcoding.si.edu , http://www.barcodeofl ife.org). At the inaugural meeting of CBOL, a Plant Working Group (PWG) was formed to explore the particular challenges of DNA barcoding in plants, more specifically land plants

IUPAC nucleotide abbreviations

Adenosine	A
Cytidine	C
Guanine	G
Thymidine	T
Uridine	U
G or A	R
T or C	Y
G or T	K
A or C	M
G or C	S
A or T	W
G or T or C	B
G or A or T	D
A or C or T	H
G or C or A	V
A or G or C or T	N
Gap of indeterminate length	-

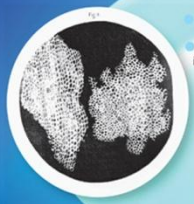
This hand-out is not an alternative of Class Lectures or Books:: FOR STUDENTS' USE ONLY

© 2015 Mirza Hasanuzzaman Also available at: www.hasanuzzaman.webs.com

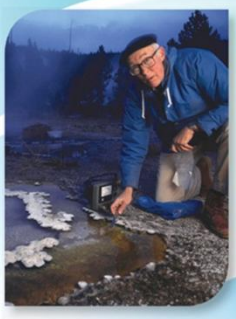
Discovering DNA FINGERPRINTING

Discovery of Cells

1665, Robert Hooke created the first drawing of cells under a microscope (shown left). 1683, van Leeuwenhoek discovered bacteria in Holland. 1833, cell nucleus discovered by Robert Brown in Scotland.



Thermus aquaticus



Bacteria that thrives at 70°C. 1969, Thomas Brock (shown) and Hudson Freeze reported the discovery of *Thermus aquaticus* found at Yellowstone National Park. DNA polymerase tolerates high temperatures, so it is ideal for PCR.

Polymerase Chain Reaction (PCR)

1983, Kary Mullis invented PCR. PCR makes millions of copies of small amounts of DNA. Uses the same DNA polymerase that cells use during division. DNA Fingerprinting is much more sensitive using PCR. Nobel Prize 1993.

"EUREKA!!! I stopped the car at mile marker 46.7 on Highway 128 (in California). Somehow, I thought, it had to be an illusion. Otherwise, it would change DNA chemistry forever. Otherwise, it would make me famous. It was too easy. Someone else would have done it and I would surely have heard of it. We would be doing it all the time."

The first DNA Fingerprint

1984, DNA Fingerprinting was invented by Alec Jeffreys in UK.
1985, first ever use in an immigration case.
1986, first forensic use in murder case.
1994, FBI DNA database formally authorized in the USA.

"**At first,** the images looked a **complicated mess**. **Then** the **penny dropped**. We had found a method of **DNA-based** biological identification."

— **Professor Alec Jeffreys,**
Inventor of DNA Fingerprinting

Genetic Material

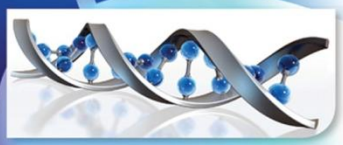
1866, monk Gregor Mendel publishes his paper on pea inheritance. Unrecognized at the time, his research led to modern day genetics.

"My scientific studies have afforded me great gratification; and I am convinced that it will not be long before the whole world acknowledges the results of my work." — Gregor Mendel

1944, DNA shown to be the genetic material in cells by Oswald Avery, Colin MacLeod, & Maclyn McCarty in USA. Controversial because most scientists then thought genes were proteins.

Electrophoresis

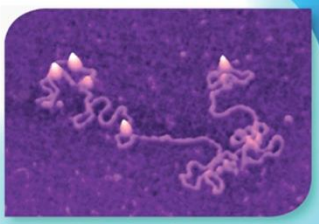
1937, Arne Tiselius of Sweden develops modern Electrophoresis. Protein electrophoresis came first, followed by DNA Electrophoresis. DNA electrophoresis is a key step in DNA fingerprinting. Nobel Prize 1948.



The Double Helix

1952, Rosalind Franklin photographed DNA with X-ray crystallography in UK leading to her discovery of the 2 forms of DNA. 1953, James Watson and Francis Crick modeled DNA as a double helix in UK. Franklin's images (shown) were controversially used in their research. Franklin died in 1958 before the Nobel Prize was awarded in 1962 to Watson, Crick, and Maurice Wilkins (who worked with Franklin).

"We have discovered the secret to life!"
— Francis Crick, 1953



Restriction Enzymes

1970, discovered by Werner Arber, Daniel Nathans, and Hamilton Smith in USA & Switzerland. Restriction enzymes cut DNA at specific sequences. Bacteria use them as a defense against viruses. Essential to genetic engineering and DNA fingerprinting. Nobel Prize 1978.

This ha[illegible] of Classroom Use. FOR STUDENTS USE ONLY

© 2015 Mirza Hasanuzzaman