GPB. 329. PRINCIPLES OF SEED PRODUCTION, SEED QUALITY REGULATION AND STORAGE (2+1)

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Practical Record Support Materials (Ex.No 7 to Ex. No 16.)

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- For WCC 75 alone 5/64" round perforated metal sieve should be used as middle sieve.

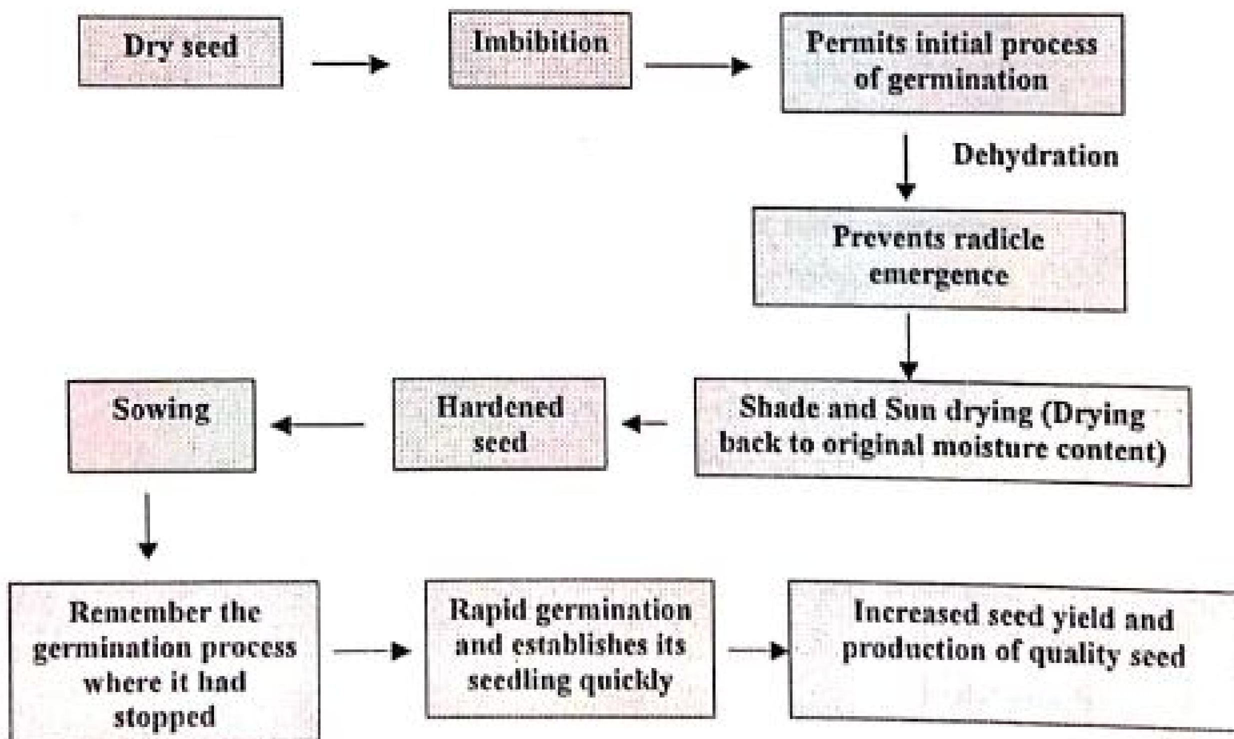
EX.No.7. SEED HARDENING TREATMENT

Seeds can be hardened for 2 purposes

- Drought tolerance
- Cold tolerance

The treatments are imposed to the seeds mainly to tolerate initial drought and cold. Cold tolerance treatment is given to germinated seeds, such treatments are given only to temperate crop and tree seeds.

Principle involved in seed hardening



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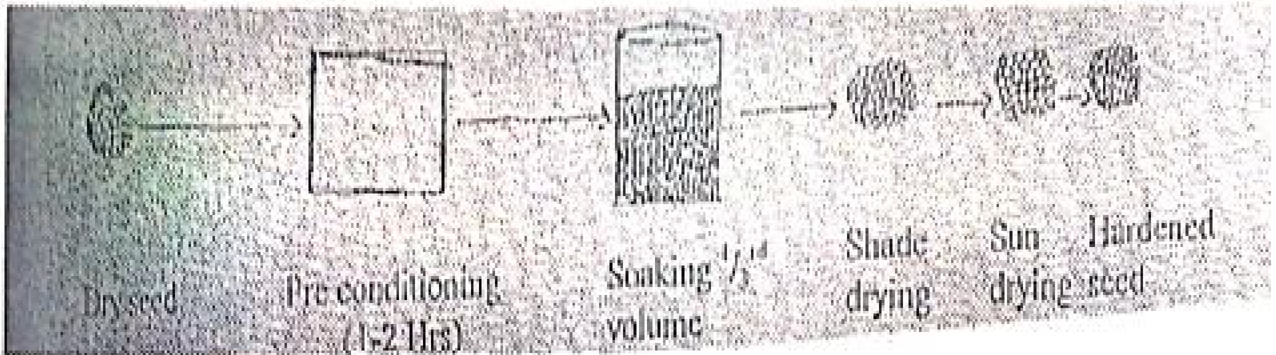
***Diagrammatic representation* (Sorghum seed hardening)**



Physiological basis for seed hardening

- Greater hydration of colloids
- Higher viscosity and elasticity of protoplasm
- Increase in bound water content
- Increase in photosynthetic activity
- Increase in hydrophilic colloids and decrease in hydrophobic colloids
- Increase in temperature requirement for protein coagulation
- More intensive respiration
- Lower water deficit
- Increase in water balance of Plants
- More efficient root system

Chemicals used for seed hardening

Aqueous solution of salts : sodium chloride, sodium sulphate, pottasium nitrate, calcium chloride, ammonium sulphate, potassium ide etc.

Growth regulators : Gibberellic acid, CCC, Kinetin, Riboflavine,Biotin , ascorbic acid

Vitamins :vit.K3, Nicotinic acid, pantothenic acid (Adanine etc.)

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Plant products : Garlic extract, coconut water

Osmatic priming D-Mannitol, polythylene glycol.

The most important factors to be considered while seed hardening are

i) Seed ; solution ratio (1:1)

ii) The duration of soaking is important

iii) Method of drying.

The effectiveness of the treatment depends upon the conduct of seed hardening process. The solution amount never be higher than the amount of the seeds. All solution added should be imbibed by the seeds. There should not be any left over solution as it causes leaching effect. Once the seeds imbibe water, the germination process takes place. At the end of soaking period the seeds should be dried back to its original moisture content. The seed when soak again the germination will be completed earlier. Whereas, for non hardened seeds germination process takes a longer duration.

Chemicals used: $CaCl_2$, KCl, KH_2PO_4, CCC (cotton)

Method of hardening the crop seeds

- Seeds are soaked in water and allowed to absorb moisture upto 35 per cent of their weight and kept in° swollen condition for about 6 hrs at about 25 C( ).lower temp. @ 15 C has been found to be more beneficial).
- Then the seeds spread in a thin layer for drying in for 2 to 3 days. The treatment is repeated or more depending upon the kind and variety of crops.
- This can also tried by using various concentrations of chemicals.
- High temperature of treatment of seed attempted for inducing resistance to drought.

Bmeficial effect of seed hardening

- Accelerate rapid germination and growth rate of seedling.
- Treeted plants recover much more quickly from wilting than those from untreated

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plants.

- Flowering is slightly accelerated in treated plants.
- Induces resistance to salinity as well as drought conditions.
- Seeds are able to with _tand higher temperature (80-105 C) for prolonged periods (24 – 46 hrs) with a loss of viablity.
- By emerging early, seedling will be able to complete more efficiently with weeds and
- Treated plants are generally better in growth.

(eg.)

Sorghum : soaking in pottasium dihydrogen 0.5 %_solution. The treatment can be given even 7 – 10 days before sowing time.

Bajra : Pre-sowing hardening treatment with Kinetin (5 ppm), chlrocholine (5ppm) and Ethrel (5 ppm) increased the dry weight accumulation and it also improves the development of stronger root system with more absorption of nutrients under dry land conditions.

Sunflower : G.A @ 500 ppm- Increase vigour germination of seedlings. Induce-early flowering by about 10 days.

Groundnut : Calcium chloride (O.5%) solution for 6 hrs Induce yield increase about 15%.

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SEED VIGOUR AND ITS MEASUREMENTS

Objective

Vigour is the stamina of the seed It is defined as the sum total of all seed attributes that express its efficacy at field level.

Characters of Good vigour seed

- Increased germination.
- Increased field emergence.
- Increased dry matter production.
- Uniform pollen production.
- Resistant to pest and disease.
- Increase of seed yield.
- Increase of seed storability.

Theories / causes for vigour loss

- Depleation of food reserve.
- Starvation of meristimatic cells.
- Accumulation of toxic materials.
- Breakdown in mechanism for triggering germination.
- Inability of ribosomes to dissociate → RNA, Proteins.
- Enzymes degradation and inactivation.
- Lipid auto oxidation.
- Formation and activation of hydrolytic enzymes.
- Fungal invasion.
- Degradation of functional structure.
- Genetic dehydration.

Ultra structural symptoms of vigour loss

- Reduced capacity for production of enzymes phospholipids, cytochrome oxidase, maleic and alcohol dehydrogenase.
- Protein synthesis get reduced.
- Increased lipid oxidation thereby production of free radicals.
- Increased vacuoles.
- Decrease in phospholipids that resulted in less membrane integrity.

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- Protoplasm detach from cell wall (Shrinkage).
- Decrease of ribosome and polysome.
- Failure of RNA and DNA synthesis due to mutation.
- Reduction in ATP & ADP production of mitochondria.
- Reduced respiration.

Manifestation Of Seed Vigour

Vigour loss occur due to mismanagement during field and storage. Vigour loss may be expressed by exhibiting symptoms. The symptoms may be divided into

1. Physical
2. Physiological
3. Performance
4. Biochemical

Physical

Based on variation weight, colour, lusture, odour, Insect damage - susceptible to invasion by microbes.

Physiological

Reduction in germination, increase of germination period, increase of abnormal seedlings, reducing in field emergence, narrower germination requirement, Reduced resistance to stress.

Performance

- Reduced growth rate,
- Ununiformity
- Slower growth rate
- Poor population
- Increased fungal infection
- Decreased seed yield
- Decreased seed storability
- Decreased pollen production

All the above are visible and measurable symptoms.

Biochemical

Reduced respiration

Reduced enzyme activity

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Reduced semipermeability
Increased free fatty acid content
Increased pro amino acid content
Increased sugar content
Increased seed leacheate
Increased toxic compounds

Seed vigour test

Various techniques are adopted in ISTA to evaluate this qualitative factor. Some of the promising tests are measurement of root length ,shoot length,drymatter production ,calculation of vigour index values (germination x total seedling length) brick gravel test, electrical conductivity test, gada test, dehydrogenase enzyme test, and percentage of amino acid, free sugars and free fatty acid.

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The main limitation of the germination test is its inability to detect quality differences among seed lots with high germination percentages. Vigour test is a more sensitive test, which aims at detecting such differences. Seed vigour is defined as "the sum of the properties, which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence". Seeds, which perform well, are termed high vigour seeds. Many methods have been developed to assess seed vigour, among them the most common methods are described below.

1. Speed of Germination

Seed lots with similar total germination often vary in their rate of germination and growth. The germination index suggested by Czabator (1962) and Djavanshir and Pourbeik (1976) for tree seeds is based on the following formula

$$\text{Germination value} = \text{Peak value of } \frac{\text{Cumulative number of normal seedlings}}{\text{Days of germination counts}} \times \frac{\text{Total number of normal seedlings}}{\text{Days of final count}}$$

2. Seedling growth test

The standard germination test only distinguishes between normal and abnormal germinants. Variations in seedling size and vigour are likely to occur within the category "normal seedling". Since initial growth is highly influenced by the seed, evaluation of seedling vigour expressed as i) dry weight or ii) evaluated as size classes, is inturn an expression of seed vigour.

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3. Conductivity Test

Low vigour seeds have been shown to possess decreased membrane integrity as a result of seed deterioration and mechanical injury. During imbibition, seeds having poor membrane structure release cytoplasmic solutes into the imbibing medium. These solutes with electrolytic properties carry an electrical charge that can be detected by a conductivity meter.

4. Accelerated ageing test

Un-imbibed seeds are subjected to conditions of high temperature (41°C) and relative humidity (around 100%) for short periods (3 to 4 days). The seeds are then removed from the stress conditions and placed under optimum germination conditions. The high vigour seed lots will show only slight decline in germination compared to low vigour seed lots.

5. Exhaustion test

It is based on the principle that seeds germinated in darkness do not carryout photosynthesis but rely entirely on nutrients derived form the seeds. The germinants become etiolated and after a specific test period, the dry weight of the seedlings is measured. Seedlings derived form high vigour seeds have the highest dry weight.

6. Cold test

Seeds are placed in soil or paper towels lined with soil and exposed to cold for a specified period, during which stress from imbibition, temperature and microorganisms occur. Following the cold treatment, the seeds are placed under favourable growth conditions and allowed to germinate.

7. Brick grit test or Hiltner test

The ability of the seeds to overcome physical stress is evaluated by germinating the seeds under a 3-4 cm thick layer of crushed brick stone or gravel.

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Importance of seed structure

1. For identification of cultivars , this can be done based on the morphological, inheritable physiological and biochemical characters
2. To decide the shelf life potential of seeds - this is possible by determining how much storage organ the seed has
3. To decide about the various post harvest operations namely drying, threshing, processing, cleaning, grading to prevent or minimize the mechanical damage
4. To design post harvest handling equipments.
5. To decide about mechanized sowing.

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Components of Seed

Seed coat - It is the outer covering of seed and gives protection. It develops from the 2 integuments of ovule. Outer layer of the seed coat which is smooth and rough is known as the testa and is formed from the outer integument. The inner layer of the seed coat is called the tegmen and is formed from inner integument.

Embryo - It is the mature ovule consisting of an embryonic plant together with a store of food, all surrounded by a protective coat, which gives rise to a plant similar to that of its mother. It is a miniature plant consists of plumule, radicle and cotyledon. The plumule and radicle without the cotyledon is known as primary axis. This is diploid (2 n) in nature.

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Radicle - Rudimentary root of a plant compressed in the embryo is the radicle, which forms the primary root of the young seedling. It is enclosed in a protective cover known as coleorhiza.

Plumule - It is the first terminal bud of the plant compressed in the embryo and it gives rise to the first vegetative shoot of the plant. It is enclosed in a protective cover known as coleoptile.

Cotyledon - Cotyledons are the compressed seed leaves. A single cotyledon (Scutellum) is present in monocots while two cotyledons are present in dicots, hence they are named as monocots and dicots, respectively. In dicots they serve as storage tissue and are well developed, while scutellum is a very tiny structure in monocots.

Endosperm - Endosperm develops from the endosperm nuclei which is formed by the two polar nuclei and one sperm nuclei. It stores food for the developing embryo. This is triploid (3 n) in nature.

Albumen - A collective term of the nutritional tissue between the embryo and the seed coat, inclusive of perisperm and endorsperm.

Perisperm - A layer of nutritional tissue of diploid maternal origin arisen from the nucellus and often surrounding the endosperm. It is usually completely absorbed before maturation but forms the principle nutritive tissue. **Eg:** Caryophyllaceae and is distinguisable in gymnosperms.

Appendages of seeds

Some seeds will have appendages that are attached to the seed coat. They vary with kind of seed. The appendages sometimes help in dispersal of seeds or in identification of genotypes. Some of the appendages are as follows :

Awn - The thorn like projection at tip of the seeds. **Eg:** Paddy - The bract tip is elongated into awn.

Hilum - It is the scar mostly white in colour present on the lateral side of the seed. It represents attachment of the seed stalk to placenta of the fruit to mother plant. **Eg:** Pulses.

Micropyle - The point where the integuments meet at the nucellar apex has been referred as micropyle.

Chalaza - At region of integumentary origin and attachment opposite to micropyle is called chalaza.

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Raphe - The area between the micropyle and chalaza is the raphe. The raphe may be visible on the seed coat of some species.

Caruncle - It is the white spongy outgrowth of the micropyle seen in some species **Eg:** Castor, Tapioca.

Aril - It is the coloured flesh mass present on the outside of the seed. **Eg:** Nutmeg.

Hairs - They are the minute thread like appendages present on the surface of the seed. **Eg:** Cotton.

Wings - It is the papery structure attached to the side of the seed coat either to a specific side of the seed coat or to all sides. **Eg:** Moringa.

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Principle

Seed extraction is the separation of seeds from fruits. Seeds are extracted from fruits for better handling of seed either for sowing or for storage. Extraction is done after harvest and before storage of seeds, but some fruits are stored as such until sowing depending on the species.

Seeds are extracted either manually or mechanically depending on the quantum of seed to be extracted. Selection of extraction type depends on nature of fruit either dry or wet fruit or indehiscent or dehiscent fruits.

Extraction Methods

Selection of extraction type depends on the nature of fruits selected for extraction as dry or fleshy fruits. Some of the widely followed extraction types are as follows

Dry extraction

- Sun or shade drying
- Exposure to high temperature
- Mechanical or manual extraction
- Seeds not extracted

Wet extraction

- Washing in deep bowls or drums, e.g. *Melia volkensii, Prunus africana*
- Washing on wire mesh screens - screens with mesh size that will retain the seeds while the pulp passes through the mesh, e.g. *Prunus* and *Vitis* spp.
- Cement mixer with abrading material. The fruits are mixed with an abrading material like gravel plus excess water and rotated in the drum for various lengths of time. While the abrading material rubs against the fruits, the pulp is gradually turnoff. E.g., *Vitex keniensis, Maesopis eminii* and *Cordia* spp.

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- Mechanical depulping - for large quantities of fruits or where the flesh tends to remain firm, this method is applicable. The depulper mechanically abrades the fruit pulp by rupturing and squeezing against or between its mechanical part
- Individual manual extraction, e.g. *Syzygium cuminii*

Biological Extraction

Fleshy/dry fruits and several indehiscent fruits types are extracted by being ingested by animals. Seeds and stones are often left cleaned and intact after ingestion although in some species a relatively large amount of seeds may be digested. E.g., *Acacia nilotica* and *A. tortilis.*

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Seed extraction techniques in tomato

Fruit grading

Based on fruit size and shape true to type fruits are selected for seed extraction and large to medium sized fruits alone to be used for extraction of higher seed recovery in tomato, . In tomato seeds are extracted from fully ripened (reddish) fruits by different methods. They are,

Fermentation method

The fruits are pulped by trampling under foot or using a pulper and collect the pulp in plastic container or cement tank. The pulp is allowed to ferment overnight. The next day seeds get separated from the pulp. The floating fraction is removed and discarded and the sinkers (Due to bacterial degradation the seed is fermented and settle down in the bottom of the container) are collected, washed well and dried in the shade and then in sun between 8 -12 Noon and 2-5 pm.

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Hydrocholoric acid method

The fruits are pulped by trampling under foot or by using a pulper and collect the pulp in a plastic container or cement tank. Add commercial hydrocholric acid @ 20ml kg⁻¹ of pulp and keep it for 30 min with occasional stirring. The seeds get separated from the pulp and sink to the bottom. The floaters can be removed and discarded. The seeds are collected, washed well with water 3-4 times and dried in shade. The advantages of this method are the seeds are attractive in colour, recovery is very high, remove the external seed borne pathogens and do not clog each other while drying. Seed quality is also very high. Seed recovery is 0.8-1.0 %.The cost of seed extraction is Rs.20/kg.

Alkali method

Seed are also extracted by alkali method and citric acid method but are injurious to seed storage.

Mechanical extraction

Tomato seeds are also extracted by using tomato seed extractor or pulper for large scale seed extraction. The seed extraction consists of two units operated by electric motor, one is fruit pulper or crusher and second one is seed and pulp separator. The whole unit is made up of stainless steel. Here extraction is immediate, seed recovery is high and pulp/ juice can be further used for making by product like jam, jelly etc., The cost of seed extraction is Rs.7.5/kg.

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Detasselling

The tool employed in hybrid seed production of maize is known as detasselling. Tassel is the male inflorescence of maize. Detasselling is removal of tassel/male flowers from the female plant. Detasselling should be done in the female parent of hybrid alone. It should be removed before anthesis and immediately after emergence. Detasselling should be completed when the tassel is well out of the boot leaf but before the anthers shed the pollen. It is done everyday from anthesis, upto 14 days.

Procedure for detasselling

The stem is to be held with left hand and the tassel is to be removed with right hand in one upward pull. The pulled tassel should be taken away from the field and burried beyond the isolation distance. In any case no spikelet should be left which may cause genetic contamination. The leaves also should not be removed as it favour reduction of seed yield.

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1. Emasculation and dusting

At the time of flower initiation in female line, the flowers that are going to open next day are selected and the petals are removed between 3-6 PM. With the help of nail or needle the total staminal (Pollen + anther + anther tube) column are removed. Then the flowers are covered with a definite colour cover for easy identification of the emasculated flower. In the morning between 9 AM - 12 noon, which is the anthesis time, the flowers of selected male parent are plugged and dusted on the stigma of the emasculated flower on opening the cover. Then it is again covered with different coloured cover to avoid pollination with other pollen and to identify the emasculated and dusted flower from the rest. The pollen from a single flower is enough to dust 4-5 female flowers. The pollen receptivity of the stigma is for 46 hours.

For easy identification of selfed boll from emasculated and dusted boll the bract can be removed while emasculating, owing to the little contribution of bract to seed set and seed yield.

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- After hand pollination, cover the crossed flowers with butter paper covers and label for identification.
- Remove all the other flower buds leaving one or two crossed flowers in a truss to ensure good fruitset.
- Allow the flowers with cover for seven days to set fruits and remove the cover after ensuring fruitset.
- Harvest fully riped fruits and extract the seeds. Crush the fruit and make it to pulp with the help of a pulper. Use plastic or cement containers.
- Add concentrated commercial Hydrochloric acid to the pulp @ 20 ml/kg of pulp and stir constantly for 30 minutes to remove mucilage.
- Wash the acid treated pull with water and collect the seeds that settle at the bottom.
- Wash the seeds repeatedly to remove the acid.
- Rapidly air dry under shade and then sun dry the seeds for 2-3 days to reduce the moisture content to 8%.
- Grade the seeds with BSS 10 x 10 wire mesh sieve and treat them with Thiram or Captan @ 2 g/kg of seeds.
- Store the seeds in aluminium foil pouch or with 150 gauge thick polyethylene lined cloth bag.

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Conventional Crossing Technique in Cotton

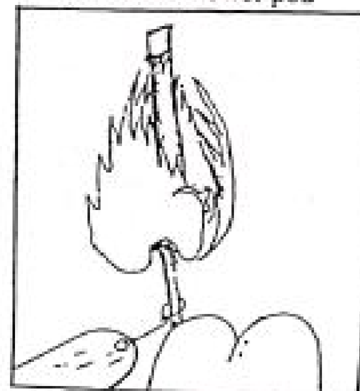
Selection of Female Flower Bud

Emasculation by Doak's method (thumb nail method)

Removal of Androecium of female flower pod

Receptive stigma on emasculated flower

Pollination with flower from male parent


Covering pollinated stigma with straw tube

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Synchronization Techniques

The flowering period of male (6- 8 days) and female (8 – 10 days) vary between the parents. For perfect seed set, synchronization of flowering between male and female is essential. This synchronization can be achieved by adopting following techniques.

PADDY

Staggered sowing

By sowing / planting the male line (early parent) in different dates. So that its flowering coincides with female. In nursery sowing of early parent (male) can be 2-3 days latter than late parent (female).

Even in main field, for continuous supply of pollen to the female, the male parent can be planted in three different dates (three seedlings). Hence the supply of pollen will be continuous and seed set will be proper.

Urea application

Apply urea at 35 kg/ha to the advancing parent (flowering delayed due to enhancing of vegetative growth by application of urea) or spray (2 – 3 sprays) of 20 kg urea in knapsack sprayer in 500 lit of water per hectare instead of power sprayer. This should be done from 4th stage of panicle initiation which is around 70 days after sowing.

Withholding of irrigation

Draining of water in 'R' line alone can delay its flowering by 2-3 days.

GA_3 application

The panicle exertion in female parent is not full. Good panicle exertion will help in improving the seed set. Spraying of GA_3 at 50 g/ha at 15-20 % flowering stage in two split doses in consecutive days with knapsack sprayer at 500 lit of spray solution per hectare will increase the seed set and final yield. Morning 8 am to 10 am and evening 5-6 pm are ideal for taking up spraying.

Rope pulling / Rod driving

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Passing of rope or rod across the population 3 – 4 times daily for 7-10 days during anthesis will supplement the pollination mechanism and aid in out crossing in hybrid seed production. The normal anthesis time is between 10 am to 1.00 pm and 3 pm to 4 pm.

SORGHUM

Different seed treatments to parental lines

The late parent may be given with hardening treatment to enhance the speed of germination and the early parent may be given with pelleting treatment to delay the speed of germination.

Staggered sowing

Based on the difference in duration of flowering of parental lines the early parent may be sown late and late parent may be sown earlier in such a way that both flower at the same time.

Application of nutrients

The urea at 1% concentration may be sprayed at primordial initiation stage (35-40 days) to the lagging parent.

Irrigation management

One irrigation is with-held to the late parent to make early flowering.

Chemical spray

Malic hydrazide 500 ppm or CCC 300 ppm is sprayed to the advancing parent at 45th day.

PEARL MILLET

The extent of synchronization problem between parents is comparatively les in cumbu than sorghum and paddy due to the tillering habit of the crop. The pollen weight is less and flying capacity is more in this crop. The pollen viability and stigma receptivity is also for longer duration owing of these factors the nicking problem is less in this crop. In the case of late parent, staggered sowing. Urea application or DAP spray or with holding of water.

REDGRAM

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The pollen parent ICPL 87109 of COPH1 hybrid should be sown one week after sowing the female parent (MST 21).

The field should be bordered with sunflower to increase the seed yield.

OILSEEDS:

SUNFLOWER

Simultaneous flowering of male and female parent is essential for effective pollination and good seed set. At times, simultaneous flowering do not happen as either of the parent is late in flowering. Best suited month of sowing for achieving better synchronization is to be identified, otherwise staggered sowing is the only convenient practical method.

COTTON

Block system of planting is adopted where female and male parents are sown separately and male flowers are plucked and dusted on female flowers which is a system of artificial supplementary pollination. Application of $ZnSO_4$ at 50 kg/ha to the soil and foliar spray of 0.5 concentration of $ZnSO_4$ on 75 and 90 days after sowing will increase the number of flower per plant. Pollen weight and pollen viability.

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MECHANICAL INJURY – SPECIAL TEST

It is required in certain crops like sunflower and paddy.

400 seeds taken from the pure seed and the number of seeds without husk are excluded (partly husk less seeds are excluded) and the % is calculated as

$$\% \text{ of huskless seeds} = \frac{\text{Number of huskless seeds}}{400} \times 100$$

This test is to identify the mechanically damaged seeds through ferric chloride test.

Basic principle

Mechanically injured areas of legume seeds turn black when placed in a solution of aric chloride.

Advantages

It is easy method for assessing the mechanical damage.

This is a practical meth0u of providing a farmer or ware house man with a quick estimation of the percent of the percent abnormalities he can expect in his crop.

Procedure

Prepare a 20% solution of ferric chloride ($FeCl_3$) by adding 4 parts of water to one of ferric chloride (Reagent lump grade) by weight. The lumps of $FeCl_3$ should be found to fine powder before preparing the solution. Count at least two 100 seed samples place them in petridishes or saucers. Pour enough solution in to each petridishes till the ends are completely submerged. Be sure that all seeds are fully submerged.

Start to separate black staining seeds with in 5 minutes after addition of the sold- regardless of how small the black stain in the seed should be separated. However be that the stain in black and not a natural dark brown. Continue to separate black seeds fifteen minutes after addition of the solution to the seeds. Do not separate the seeds the fifteen minutes are up. The solution should be poured off, at the conclusion of the and saved for reuse. Count the number of black stained seeds. The percentage of the damaged seed should be worked out. The lower the percent of the black seed, quality of the and is better.

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6. DETERMINATION OF MOISTURE CONTENT OF SEEDS-HOT AIR OVEN METHOD

The moisture content of seed is an important determinant of the duration of seed viability. High seed moisture content at harvest and later during storage, the high moisture content of seed decreases viability more rapidly because of mould growth, heating damage, ageing and greater insect damage. Hence it is important that to know the seed moisture content immediately after harvest and also after drying during before storage.

The sample sent for moisture testing should be handled in such a way to conserve the initial moisture content of seed. The seed should be packed in a moisture proof container (metal or plastic) submitted to the seed testing station without delay and the same should be analysed immediately upon arrival. The seed should be exposed to the atmosphere as little as possible.

Objective

The objective is to determine the moisture content of seed by methods suitable for routine use.

Definition

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

Principle

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.

Apparatus required

The following apparatus are required

i) an adjustable grinding mill /mixie
ii) constant temperature oven and accessories which shall include containers and a desiccator
iii) Sieves
iv) Analytical balance

Moisture Determination Methods

1. Air oven method
   a) Low constant temperature oven
   b) High constant temperature oven
2. Infra red moisture meter

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3. Seed moisture meter
4. Electronic moisture meter

In case of methods 2,3 & 4 equipments are used to determine the moisture content very quickly but the accuracy level some what lesser when compared to the air oven method because these meters cannot read the moisture loss very precisely. For accuracy each meters used for quick determination must be calibrated for each species against the standard air-oven method. The moisture determination should be executed under standardized conditions.

PROCEDURE

* The moisture analysis are carried out on independently drawn duplicate sample .
* The sample should be weighed to an accuracy of 1 mg, if pre drying is required it should be done
* Each empty container should be weighed with its cover . The standard sample should be mixed with a small spoon and two portions of 5 gm each are weighed along with the containers. While filling we should take care that the seed should be distributed evenly over the bottom of the container.
* After weighing the containers are placed on top of their covers on the tray in the pre-heated oven (oven heated to the prescribed drying temperatures)
* After drying the containers are closed with their covers and it should be allowed to cool for 30 minutes in a dessicator and weighed again. The moisture content is calculated as follows.

$$M = \frac{M2 - M3}{M2 - M1} \times 100 = \frac{\text{Loss in weight}}{\text{Initial weight of seed}} \times 100$$

M1 - Weight of empty container with cover
M2 - Weight of container with cover and seeds before drying.
M3 - Weight of container with cover and seeds after drying

The results of the duplicate determination should not be defer by more than 0.2 % otherwise, the analysis should be repeated in duplicate.

Hot air oven method

a) Low constant temperature oven method

The working sample taken for moisture estimation must be evenly distributed over the surface of the container . Weigh the container and its cover before and after filling. Place the container rapidly in an oven maintaining at a temperature 103+ 2

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C and dry for 17 + 1 hrs. At the end of the prescribed period cover the container and place it in a desiccator and cool for 30 to 45 minutes. After cooling weigh the container with its cover and contents. The relative humidity of the ambient air in the laboratory must be less than 70% when the determination is carried out .

b) High constant temperature oven method

The procedure is same that of low constant temperature oven method but here the oven is maintained at a temperature of 130 - 133 C , the sample should be dried for a period of 4 hours for zea mays two hours for other cereals and one hour for other species.

Precautions to be taken during moisture estimation

Selection of containers

The containers selected for moisture determination should be made upof non-corrosive metal and it should approximately 0.5 mm thickness with sides ro-nded at the base having flat bottom level edges and should have loose fitting cover. The container may be made up of metal or glass. The container should have 3 cm height and should not have more than 8 cm diameter. The size of the sample used for moisture estimation vary with the diameter of the container.If it is

less than 8 cm diameter	- 4-5gms
8 cm diameter or large	- 10 gms

Grinding and Predrying

Large seeds must be ground before drying e.g., for leguminous seeds only coarse grinding is necessary at least 30% of the ground material should pass through a wire sieve with 1 mm meshes . Some seeds like cotton and cereal seeds require fine grinding that is atleast 50% of the ground material must be able to pass through a wire sieve of 0.5 mm mesh sieve and not more than 10 % should remain on a sieve with 1 mm meshes.

If the seed sample requires grinding and the moisture content exceeds 17% (are 10% in soyabean and 13% in rice) Pre drying is necessary before grinding. For this two 50 gms sample should be weighed and placed on two open trays at 130 C for 5 to 10 minutes. Very moist seed about 25% moisture or greater are spread on two open trays and should be dried at 70 C for 2 to5 hours . The drying time depends on the initial water content of seed. Then the trays should be left uncovered in the lab for about 2 hours . If the relative humidity of the laboratory

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exceeds 70% it is preferable to put the pre dried seeds in a closed desiccator. After that the two samples are weighed and to be used for grinding. The ground material is then subjected to a moisture test using the air oven method. The moisture content M is calculated by using the following formula

$$M = S1+S2 - \frac{S1xS2}{100}$$

S1 - Percentage of moisture lost by pre drying (stage 1)
S2 - Percentage of moisture lost by the oven method(stage 2)

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 Healthy and viable seed is the first pre-requisite for increasing seed production and to reduce possible seed crop failures. Until and unless we do not know the health status of the seed, it is not possible to manage the disease. To know health of the seed, different testing methods for different pathogens / diseases of different crops have been developed.

Testing methods for seed borne fungi / diseases

1. Examination of dry seeds

It is applied for detection of seed borne fungal pathogens which cause dicolouration of the seed or change the shape and size of the seed. Also for detecting fungal structures present on or within seed.

Procedure: Working sample 2000 seeds. All parts of seed sample are examined carefully by naked eye for the presence of discolouration and fungal structures and non seed material are removed and identified. e.g., Karnal bunt of wheat ***Neovossia indica,*** Ergot of bajra ***Claviceps fusiformis.***

2. Washing test

This method is used particularly for smut and fungi in gramineous hosts except loose smut of wheat and barely. It can also be used for downy mildew (***Pernospora manshurica***) of soybean and tumour disease (***Protomyces macrosporus***) of coriander.

Procedure

Sample taken by weight / number of seed and put in conical flask containing sufficient water. The flask is shaken for 5-10 minutes. Drops from the washing water are examined under microscope for identification of fungal spores.

e.g., Flag smut of wheat - ***Urocystis agropyri,*** Smut of pearl millet - ***Tolyposporium penicillariae***

3. NaOH seed soak method

Applied for Karnal bunt of wheat and bunt of rice.
Procedure: Working sample - 2000 seeds.

Seeds are soaked in 0.2 % NaoH for 18-24 hr, at 20-25°C. After this swollen seeds are spread over blotter paper to remove excess water / moisture. Infected seeds giving jet-black appearance can be separated from healthy seeds.

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4. **Blotter method**

This method is widely used. All kinds of cereals, vegetables, crucifers, legumes, ornamentals and forest seeds are tested by this method.

Procedure

Seeds are placed on well water soaked filter paper and incubated at 20± 2°C usually for 7 days in alternating cycles of 12 hr light and 12hr darkness. Then individual seed is examined under stereo-microscope and fungi are identified based on growth characters and sporulation.

In fast germinating seeds 2,4-D (2,4-dichlrophenoxy acetic acid) @ 0.10 to 0.20 per cent solution is used to check the growth of the seedlings.

In case of cereals this can be replaced by deep freeze blotter method (10°C for two days, then at 20°C for four days, then at 20°C for overnight and at 20°C for five days).

e.g., Black and gray leaf - ***Alternaria brassicicola***, Spot of crucifers - **A. *brassicae*,** Ascochyta blight of gram ***Ascochyta rabiei.***

5. **Agar plate method**

This method is used for detection of same type of pathogens as in blotter method. Those fungi which are not easily detectable in blotter method can be detected by this method.

Procedure : Working sample - 400 seeds

Seeds are planted on specific medium after treating with 1 to 2% sodium hypochlorite (NaOCl) and incubated in the same way as in blotter method. Fungi are identified based on colony characteristics. Colonies with doubtful identity should be examined under compound microscope.

e.g,

Pathogen	Medium
Alternaria triticnia	PDA
Fusarium oxyporum	PDA
Xanthomonas, Pseudomonas	Nutrient agar

6. **Seedling symptom test**

This test is applicable for those fungi which are capable of producing symptoms on the root and shoot of the young seedlings. This test for certain pathogens provides information pertaining to field performance of the seed lot.

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Procedure

Seeds are sown in autoclaved soil or sand or any type of other media and incubated at 20°C for 14 days under 12h of alternating cycles of artificial light and darkness. After incubation, individual seedling is examined and per cent infection is calculated. e.g., ***Alternaria*** spp. in crucifers and wheat, ***Fusarium*** spp. in a number of hosts.

7. Embryo count method

This method is specifically used to detect loose smut of wheat and barely. Downy mildew (***Seclerospora graminicola***) of bajra can also be detected by this method.

Procedure

2000 seeds are soaked in 5 per cent solution of NaOH and 0.01 per cent (100 ppm) trypan blue solution for 24 h at 25-30°C. Pass the material through different sieves of 3.5, 2.0 and 1.0 mm size along with showers of tap water. Dehydrate the embryos with methylated spirit or 95 % ethyl alcohol for 2-3 minutes. Transfer the embryos in 200 ml of lactic acid + glycerol + water mixture (1:2:1). After that transfer the embryos into a 250 ml beaker containing 75 ml of lactic acid + 150 ml glycerol (1:2) and then embryos are boiled for 2 minutes. Then the mixture is allowed to cool down. Observe the embryos under steromicroscope for the presence of mycelium. Calculate the per cent infection.

8. Non destructive seed health test

This test is conducted on high valued germplasm that can not be sacrificed as in conventional method. This test is easily applicable in large seeded crops such as corn, soybean and common bean, however, it can also be applied in small seeded crop like alfalfa, cabbage and lettuce. It consists of extracting tissue from dry seed with a metallic drill or cork borer (1 to 3 mm) and testing extracted tissue for the disease. This test does not decrease germination rate and also help in detection of the disease. e.g., ***Ustilago segetum*** in wheat, ***Phoma betae*** in beet

9. Fluorescence method

The fungus to produce a fluorescent substance under NUV light. e.g., ***Ascohyta pisi*** in pea seeds exhibits yellow green fluorescence.

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GROW OUT TEST

Objective

To determine the genetic purity of a given seed lot of a released cultivar and the extent to which the submitted sample conforms to the prescribed standards.

Sampling

The samples for grow-out test are to be drawn simultaneously with the samples for other quality tests and the standard procedure shall be followed.

The size of the submitted sample will be as follows;

- 1000grams: for Maize, Cotton, Groundnut, Soybean, and species of other genera with seeds of Similar size.
- 500grams: for Sorghum, Wheat, Paddy and species of other genera with seeds of similar size
- 250grams: For Beta and species of other genera with seeds of similar size.
- 100grams: For Bajra, Jute and Species of all other genera.
- 250 tubers/planting stakes/roots/corms - Seed potato, sweet potato and other vegetatively propagating crops.

III. Procedure

While raising a test crop, standard and recommended agronomic/cultural practices (e.g., field preparation, size of the plot, row length, distance between rows, distance between plants, irrigation, fertilization etc.), in respect of individual crops are to be followed both for the unknown sample and its control.

The possibility to prove the genuineness of a cultivar by grow-out test is based on hereditary characteristics of the plants. Usually the cultivar differences are more distinct if growth conditions are favourable. Crop should be so grown that the genetical differences express themselves as clearly as possible. In self-fertilizing species the individual of a cultivar may be theoretically identical whereas the individual of a cultivar in a cross-fertilizing species may not be genetically similar, but comprise a number of types. Therefore,

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it is easier to determine the cultivar purity in self-fertilizing species than in cross-fertilizing species where the examination of greater part is based on the mutual comparison between the sample to be tested and the standard sample. Hence, it is essential to sow the various samples of the same cultivar in succession and standard samples are sown at suitable intervals (for example one standard sample for every ten samples to be tested). The size of plots, row length etc. will differ crop to crop. However, the specifications for different crops are indicated in the following table. The Certification Agency may change the specification if considered necessary.

Sl. No.	Crop	Row length (m)	Plant to plant distance (cm)	Space between rows (cm)	Space between plots (cm)	No. of replications
1.	Wheat, barley, oats	6	2	25	50	2
2.	Pea, cowpea	6	10	45	90	2
3.	Chickpea, greengram, blackgram	6	10	30	60	2
4.	Maize	10	25	60	90	2
5.	Hybrid cotton	5	10	45	45	2
6.	Paddy					
a.	Very early to medium	6	15	20	45	2
b.	Late and very late	6	25	30	60	2
7.	Pearl millet	6	10	60	90	2
8.	Sorghum	6	10	45	60	2

The seed rate may be adjusted depending on the germination percentage of individual sample and the sowing may be done by dibbling. Subsequent thinning is not recommended.

The test crop may be raised along with the control either in all the areas recommended for the variety or in off-season nurseries. The authentic control sample from the originating plant breeder/breeding institute is to be maintained by the testing station / agency following standard procedures. A minimum of two hundred plants from control sample will be raised along with the test crop.

IV. Observation

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Standard	Reject numbers for sample sizes of	
	800	400
99.5 (1 in 200)	8	*
99.0 (1 in 100)	16	8
95.0 (5 in 100)	48	24
90.0 (10 in 100)	88	44
85.0 (15 in 100)	128	64

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