# PRACTICAL Manual

**on**

# Principles of Seed Technology

**AST 241 3(1+2)**

**(For Undergraduate Agricultural students)**

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**2020**

**Department of Genetics & Plant Breeding**
**College of Agriculture**
**RANI LAKSHMI BAI CENTRAL AGRICULTURAL UNIVERSITY**
**Jhansi-284003**

**Syllabus [AST 241 3(1+2)]**
Seed production in major cereals: Wheat, Rice, Maize, Sorghum, Bajra and Ragi. Seed production in major pulses: Urd, Mung, Pigeonpea, Lentil, Gram, Field bean, pea. Seed production in major oilseeds: Soybean, Sunflower, Rapeseed, Groundnut and Mustard. Seed production in important vegetable crops. Seed sampling and testing: Physical purity, germination, viability, etc. Seed and seedling vigour test. Genetic purity test: Grow out test and electrophoresis. Seed certification: Procedure, Field inspection, Preparation of field inspection report. Visit to seed production farms, seed testing laboratories and seed processing plant.

Name of Student .....

Roll No. .....

Batch .....

Session .....

Semester .....

Course Name: .....

Course No.: .....

Credit .....

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# CERTIFICATE

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Date: Course Teacher

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**Exercise No. 1**

## Objective: To Study about seed sampling procedure

**Problem 1:** Write about different types of seed triers and draw their diagrams.

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**Problem 2:** Write about different type of seed dividers and draw their diagrams.

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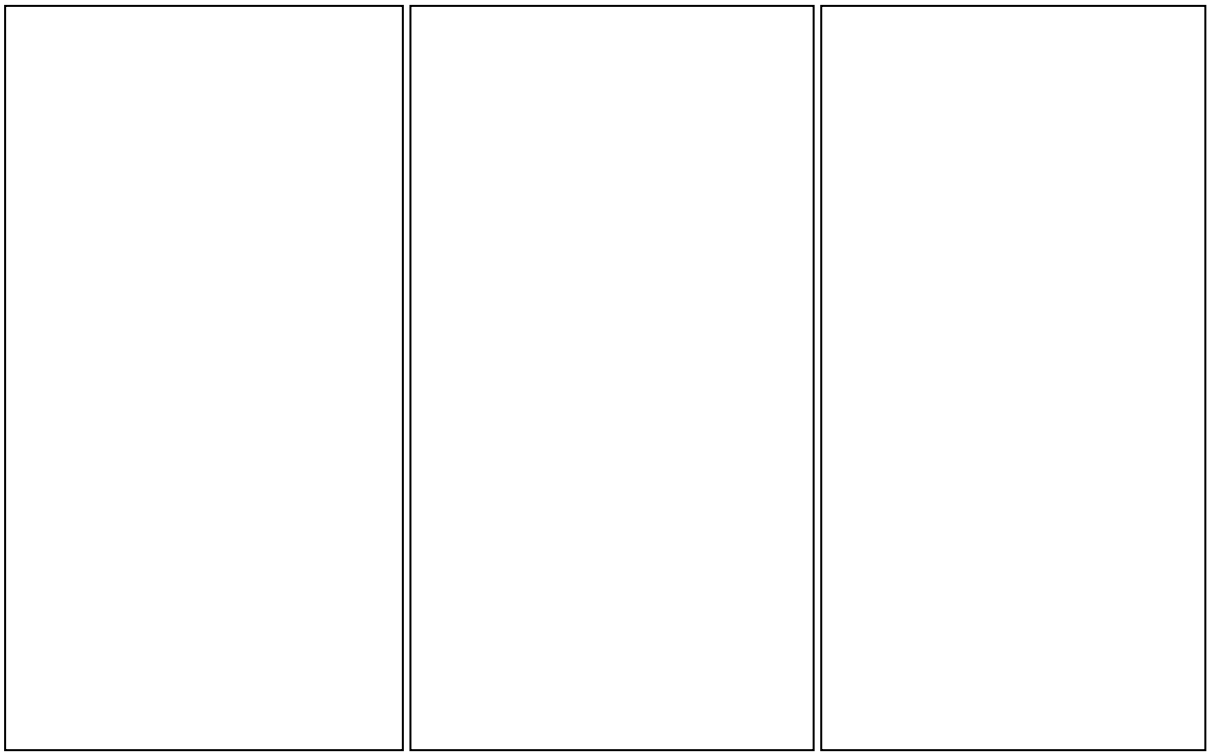

**Problem 3:** Write down the method of deriving primary, composite, submitted and working sample.

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**Exercise No. 2**

## Objective: To analyse physical purity by number

**Problem 1:** Analyse the physical purity of the given submitted seed sample by number
**Materials required:** ……

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**Diaphanoscope:** ……

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**Procedure:**……

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**Result:** **……**……

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**Exercise No. 3**

## Objective: To analyse physical purity by weight

**Problem 1:** Analyse the physical purity of the given submitted seed sample by weight
**Materials required:** ……

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**Procedure:**……

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**Calculation:** ……

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**Result:** ……

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## Exercise No. 4

## Objective: To test the germinability of a seed lot by paper towel method.

**Problem 1:** Determine the germinability of given seed lot by paper towel method
**Materials required:** ……

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**Procedure:**……

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**Observation:** ……

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**Result:** ……

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**Calculation:** ……

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**Conclusion:** ……

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**Exercise No. 5**

## Objective: To test the germinability of a seed lot by sand method.

**Problem 1: D**etermine the germinability of given seed lot by sand method
**Materials required:** ……

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**Procedure:** ……

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**Observation:** ……

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**Result:** ……

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**Calculation:** ……

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**Conclusion:** ……

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## Exercise No. 6

## Objective: To test the germinability of a seed lot by filter paper method.

**Problem 1:** Determine the germination percentage of given seed sample using filter paper method
**Materials required:** ……

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**Procedure:** ……

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**Observation:** ……

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**Result:** ……

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**Calculation:** ……

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**Conclusion:** ……

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**Exercise No. 7**

## Objective: To determine the viability of given seed sample

**Problem 1:** Determine the viability of given seed sample.

**Principle:** ……

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**Reagents:**……

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**Equipment:** ……

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**Procedure:**……

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**Evaluation:** ……

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**Observation:**……

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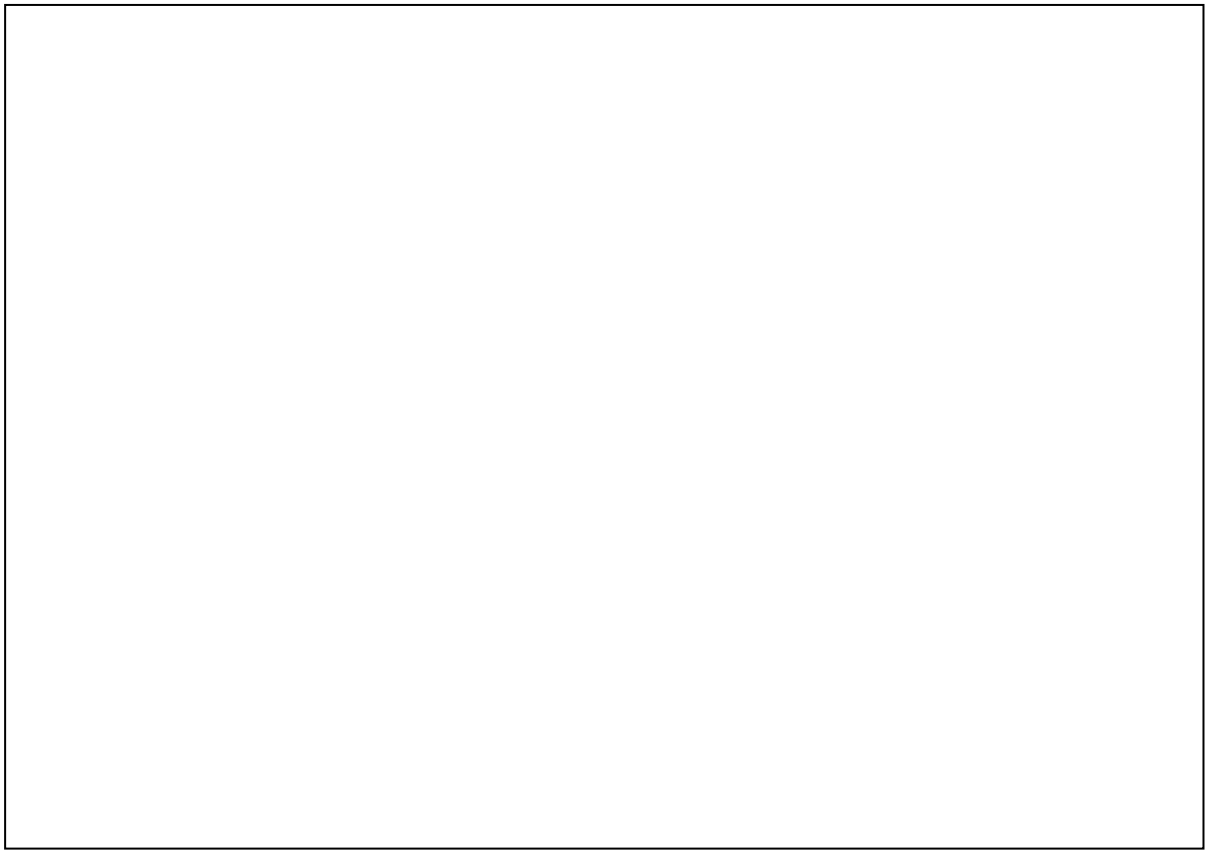

**Exercise No. 8**

**Objective: To determine the moisture content of seed sample using universal moisture meter**

**Principle:** ……

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**Equipment:** **.....**……

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**Procedure:**……

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**Observation:** ……

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**Calculation:** ……

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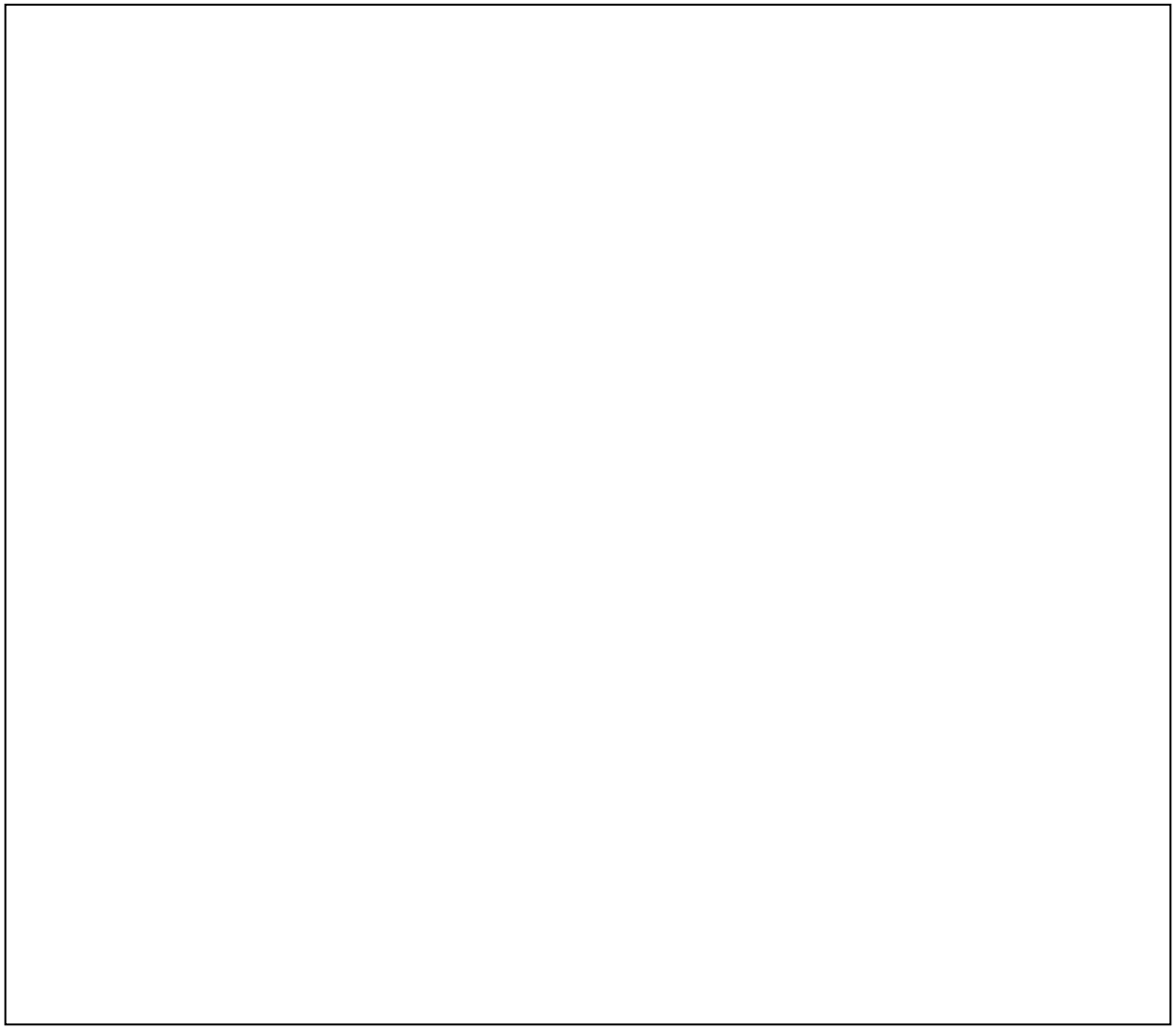

## Exercise No. 9

## Objective: To determine vigour of the given seed sample by paper piercing test

**Problem 1:** Enlist different methods of seed vigour testing

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**Problem 2:** Determine the vigour of given seed sample by paper piercing test

**Materials required:** ……

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**Procedure:**……

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**Observation:** ……

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**Result:** ……

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## Exercise No. 10

**Objective: To determine the vigour of the given seed sample by brick gravel test**

**Materials required:** ……

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**Procedure:** ……

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**Observation:** ……

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**Result:** ……

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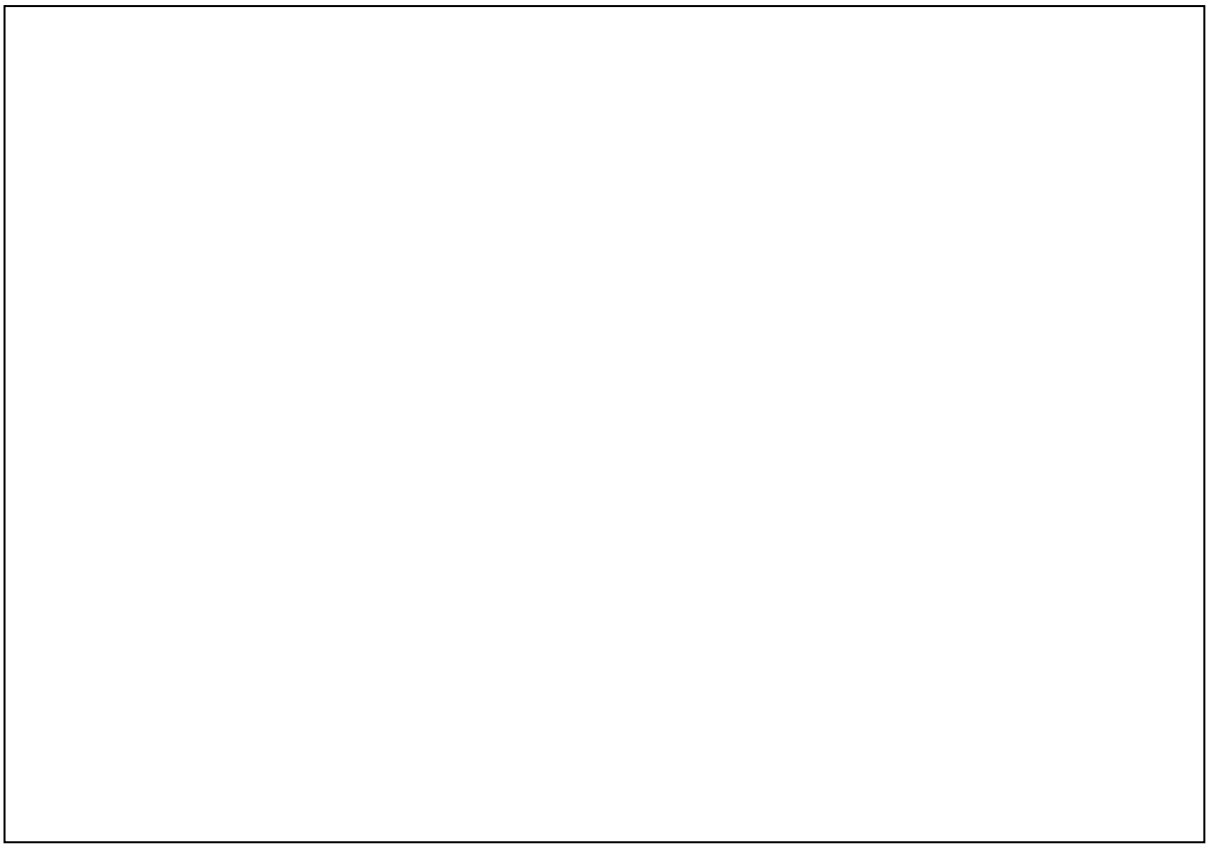

**Exercise No. 11**

## Objective: To determine genetic purity at seed level.

**Problem:** Determine the genetic purity of given seed sample at seed level.
**Equipment required**: ……

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**Procedure:**……

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**Observation:** ……

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**Result:** ……

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**Exercise No. 12**

## Objective: To determine genetic purity by grow out test

**Sampling:** ……

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**Land requirements:** ……

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**Observation:** ……

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**Calculation:** ……

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**Result:** ……

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**Conclusion:** ……

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**Exercise No. 13**

## Objective: To determine genetic purity of given sample by phenol colour test.

**Problem 1:** Enlist different biochemical methods for determination of genetic purity.

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**Problem 2:** Determine the genetic purity of the given seed sample by phenol colour test.

**Reagents and equipment: ……**……

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**Procedure**: ……..……..……...

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**Observation:** ……..……

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**Result:** ……..……...……..

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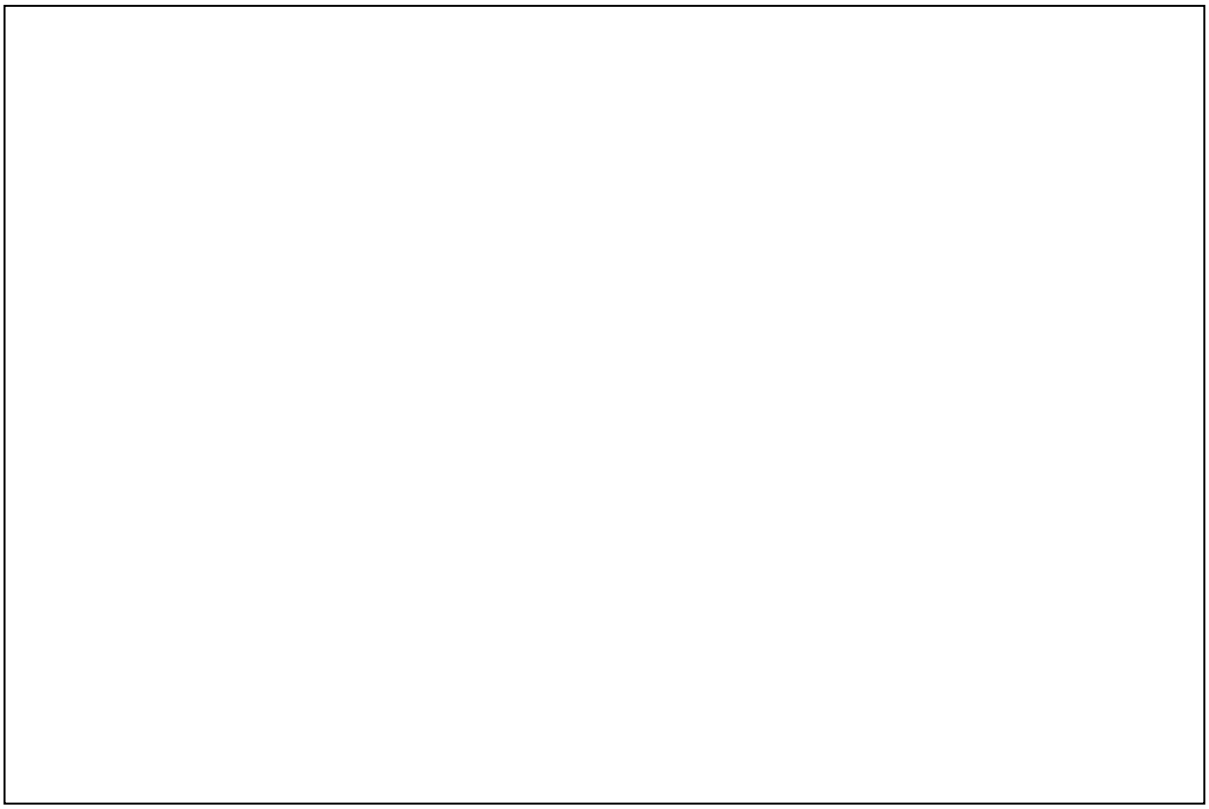

**Exercise No. 14**

## Objective: To determine genetic purity of given sample by KOH bleach test.

**Problem:** Determine the genetic purity of the given seed sample by KOH bleach test.
**Reagents and equipment: ……**……

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**Procedure:**……..……..……...

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**Observation:** ……

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**Result: ……**

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## Exercise No. 15

## Objective: To determine genetic purity of given sample by peroxidase activity test.

**Problem:** Determine the genetic purity of the given seed sample by peroxidase activity test.
**Reagents and equipment: ……**……

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**Procedure:**……..……..……..

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**Observation:** ……

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**Result: ……**

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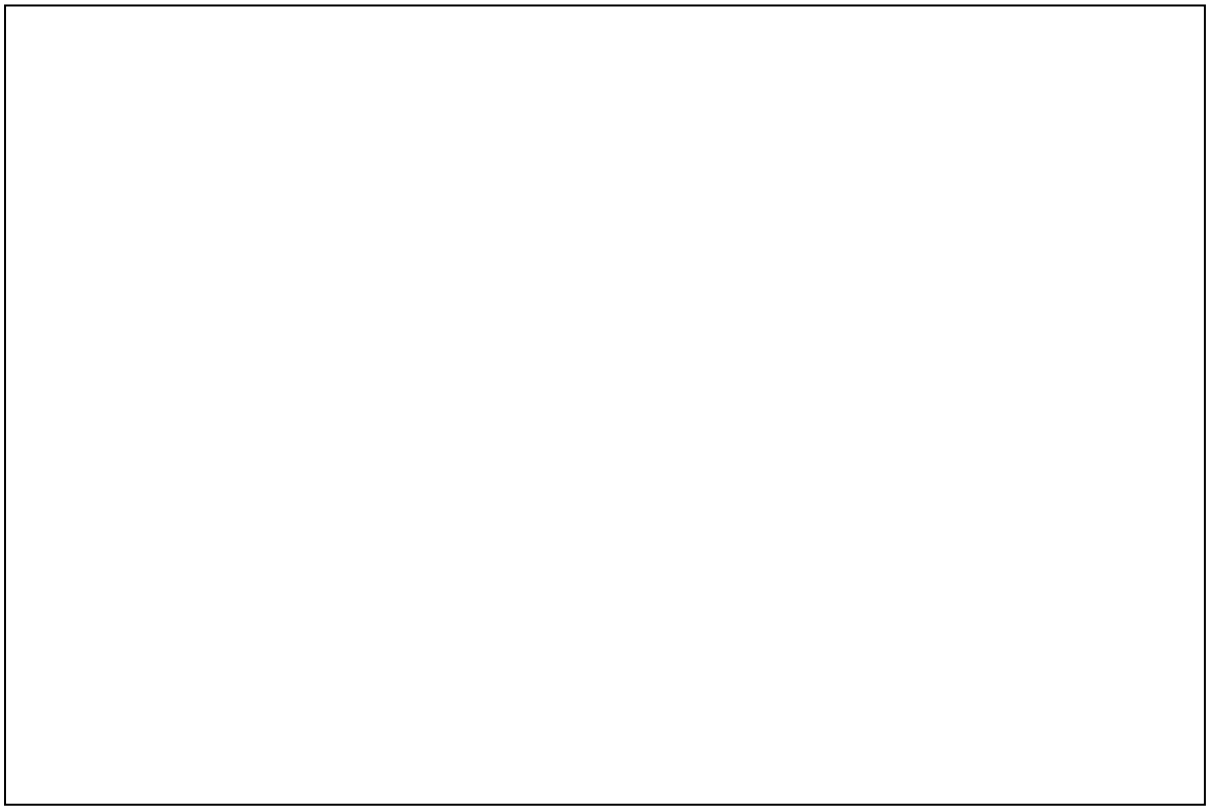

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## Exercise No. 16

## Objective: To test genetic purity by electrophoresis method.

**Problem:** Determine genetic purity of given seed sample by electrophoresis method.

**Principle:** ……

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**Equipment:**……

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**Sample preparation:**

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**Protein extraction:** ……

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**Gel preparation:** ……

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**Filling of cassette:** ……

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**Electrophoresis:** ……

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**Interpretation:** ……

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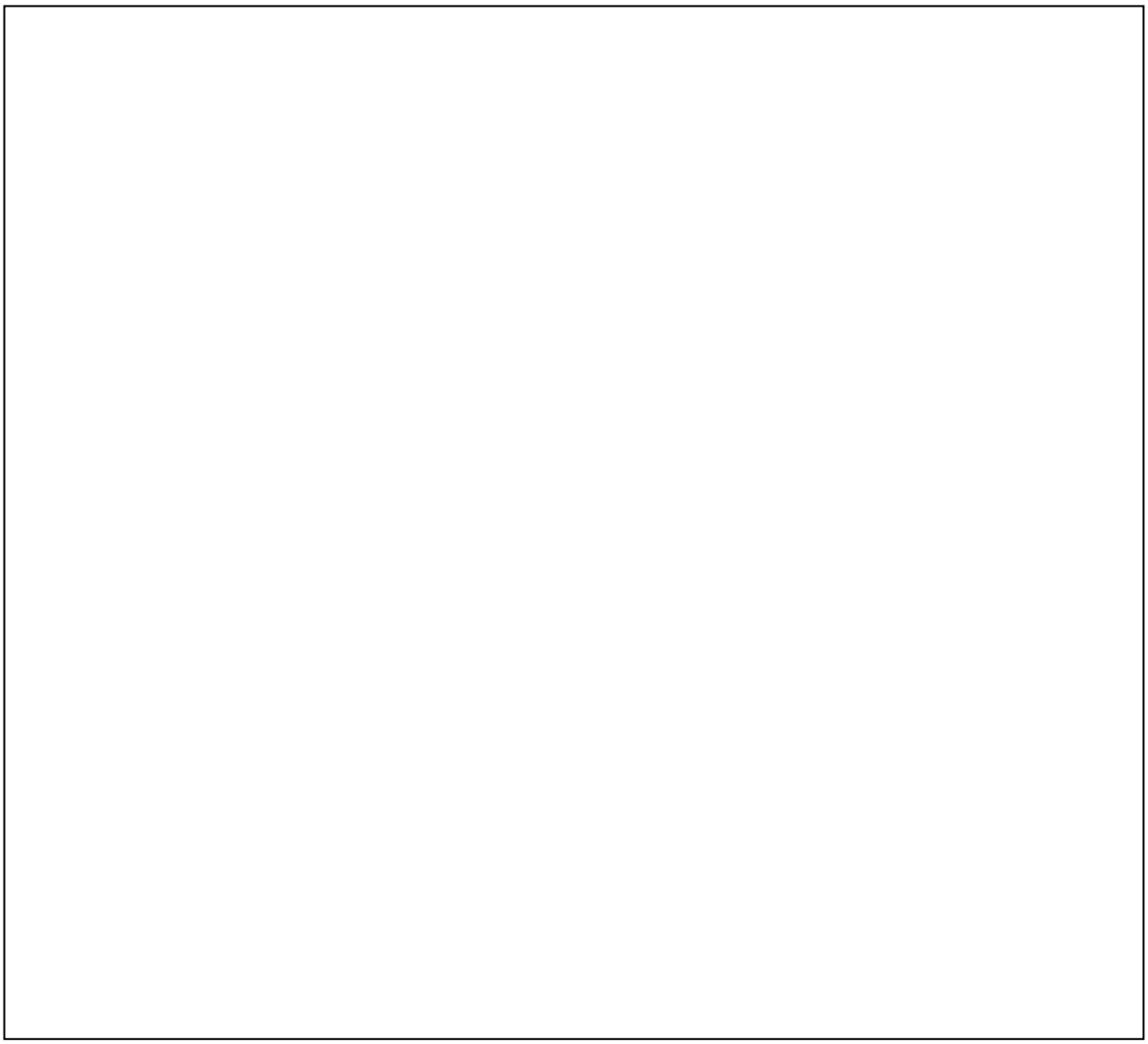

## Exercise No. 17

## Objective: To study seed production techniques in rice.

**Problem:** Write down the isolation requirements for paddy crop. Discuss the methods of hybrid seed production in rice, its isolation and planting ratio.

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## Exercise No. 18

## Objective: To study seed production techniques in wheat and barley.

**Problem:** Discuss the cultural practices and isolation requirements for seed production of wheat and barley.

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## Exercise No. 19

## Objective: To study seed production techniques in minor millets.

**Problem:** Discuss cultural practices and isolation requirements for seed production of minor millets.

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## Exercise No. 20

## Objective: To study seed production techniques in sorghum.

**Problem:** Write down the isolation requirements of open pollinated sorghum varieties seed production. Discuss the hybrid seed production in sorghum, its isolation and planting ratio

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## Exercise No. 21

## Objective: To study seed production techniques in pearl millet.

**Problem:** Write down the isolation requirements of open pollinated pearl-millet varieties seed production. Discuss the hybrid seed production in pearl-millet, its isolation and planting ratio.

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## Exercise No. 22

## Objective: To study seed production techniques in maize.

**Problem:** Write down the isolation requirements of open pollinated maize varieties seed production. Discuss the hybrid seed production in maize, its isolation and planting ratio.

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## Exercise No. 23

## Objective: To study seed production techniques in pigeon pea.

**Problem 1:** Draw a table of isolation requirements for seed production in major pulses.

**Problem 2:** Discuss the method of seed production techniques in Pigeon pea.

**Exercise No. 24**

## Objective: To study seed production techniques in gram.

**Problem:** Discuss the method of seed production techniques in gram.

**Exercise No. 25**

## Objective: To study seed production techniques in black gram and green gram.

**Problem:** Discuss the method of seed production techniques in black gram and green gram.

**Exercise No. 26**

## Objective: To study seed production techniques in pea.

**Problem :** Discuss the method of seed production techniques in pea.

**Exercise No. 27**

## Objective: To study seed production techniques in groundnut.

**Problem 1:** Draw a table of isolation requirements for seed production in major oilseeds.

**Problem 2:** Discuss the method of seed production techniques in groundnut.

## Exercise No. 28

## Objective: To study seed production techniques in sunflower.

**Problem 1:** Discuss the method of hybrid seed production techniques sunflower.

**Exercise No. 29**

## Objective: To study seed production techniques in sesame.

**Problem:** Discuss the method of seed production techniques in sesame.

## Exercise No. 30

## Objective: To study seed production techniques in rapeseed and mustard.

**Problem:** Discuss the method of seed production techniques in rapeseed and mustard.

**Exercise No. 31**

**Objective: To study the method of hybrid seed production in cotton.**

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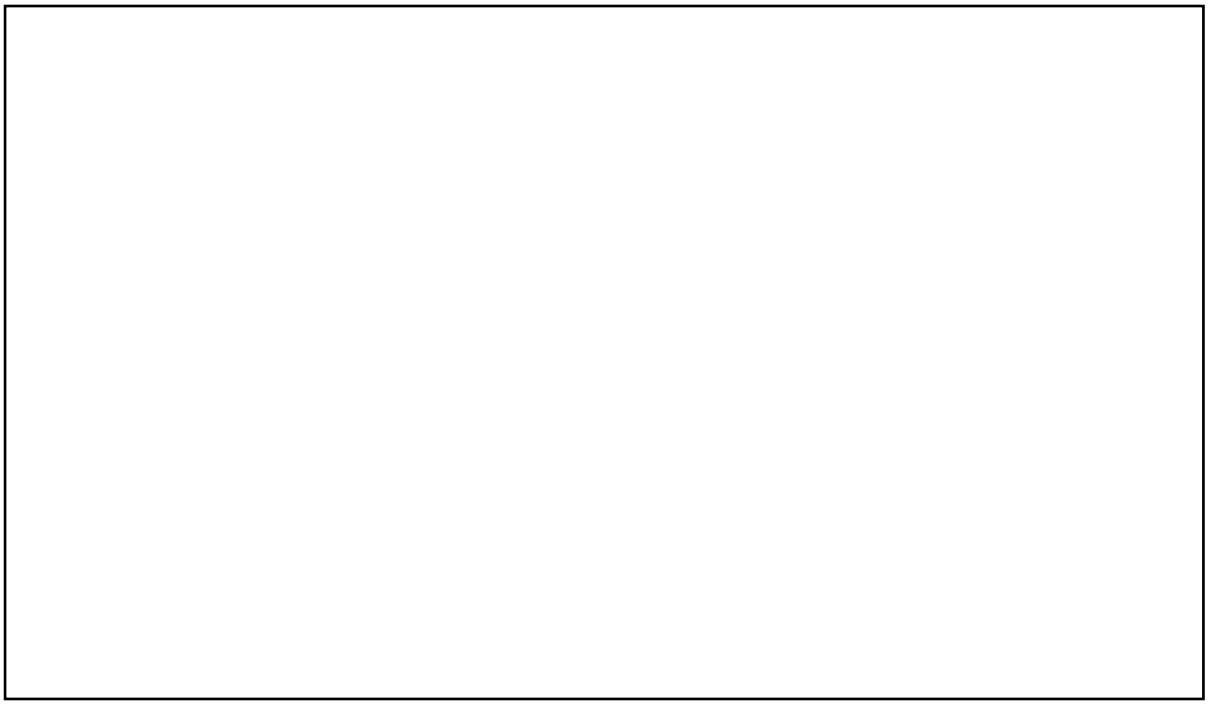

**Exercise No. 32**

**Objective: To study the hybrid seed production in tomato and brinjal.**

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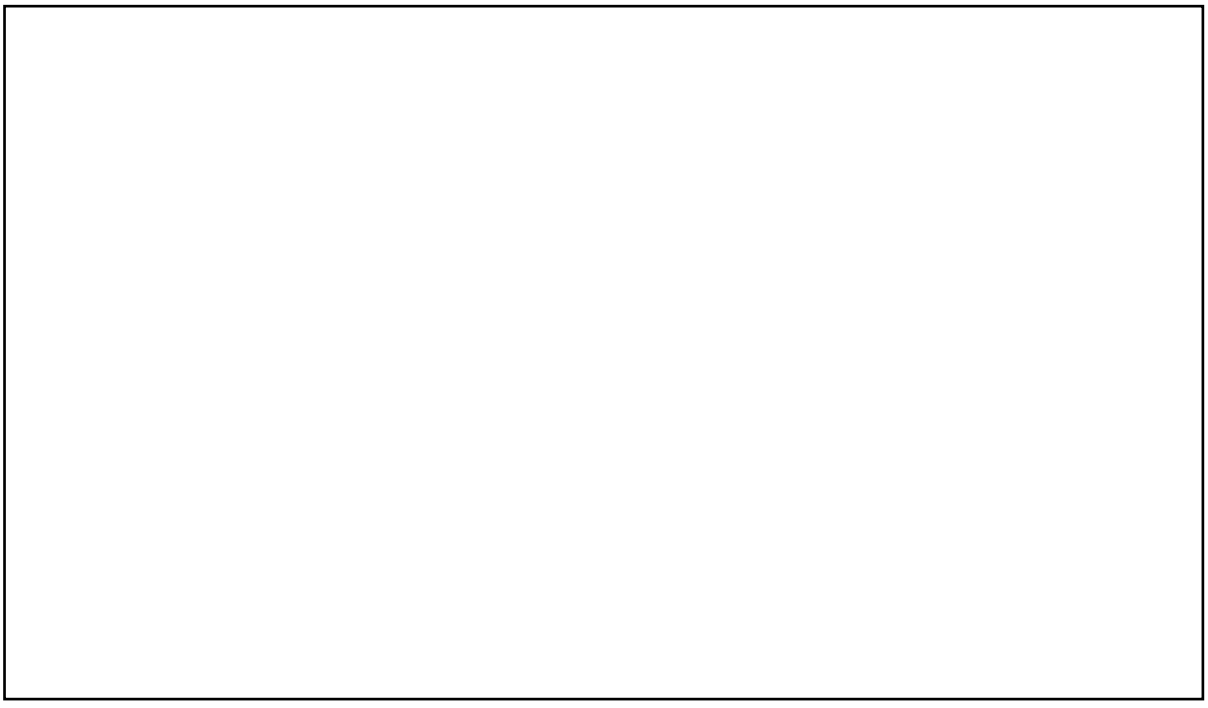


**Exercise No. 33**

**Objective: To visit of seed production farm.**

**Exercise No. 34**

**Objective: To visit seed testing laboratories and seed processing farms.**

## SEED SAMPLING PROCEDURES

Samples are derived from different portions of a seed lot and mixed to obtain a sample of required quantity representing the seed lot in true sense.

**Equipment and materials-** Trier, plastic tubes, bags, balance, seed divider, sticker and labels

**Trier-** Required drawing the primary sample from the seed lot stored in bags or containers. Two types of triers are required for sampling

**Stick trier-** Made up of two hollow steel tubes with coinciding slots. Slots can be closed and opened by turning the inner tubes within the outer tube with the help of handle. Inner tube is divided into compartments by transverse partition whereas outer tube has a solid end.

**Nobbe trier-** Long hollow metal with a sharp solid pointed end and an oval hole just near the pointed end.

**Seed divider-** Used for getting desired quantity of true to type seed sample for testing.

**Boerner type divider-** It has a funnel shaped hopper on the top with valve at exit end. Just below the hopper a cone with its upwards upward pointed end is present with numerous closely spaced channels at the circumference of the cone. Alternate port leads through a common pout among the two. Two pans are provided to collect seeds from spouts.

| | | | |
|---|---|---|---|
|  |  |  |  |
| TRIER | BOERNER TYPE DIVIDER | SOIL DIVIDER | GAMET DIVIDER |

**Soil divider-** It consist of a hopper with rectangular ports on the lower end fixed in straight row. Alternate port leads to left or right in receiving pan with the help of ducts.

**Gamet divider-** It consists of a hopper, spinner and electric motor. The periphery is divided into ports and half of the ports are connected with one spout and remaining with second.

**Procedure-**

**Primary sample-**Drawn from different portions or depth by inserting the stick trier diagonally in the seed bag at an angle of 30 degree with the hole present at pointed end facing downwards. The spear is withdrawn gently so that equal quantity of seeds enters into the hole from the centre to the size of bag.

**Composite sample-** Primary samples drawn from different places of a lot are mixed to from composite sample. The size should be 10 times more than the required submitted sample.

**Submitted sample-** The composite sample is mixed thoroughly and reduced up-to desired quantity with the help of seed divider or by repeated halving method.

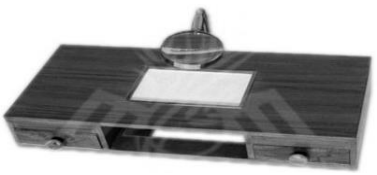
**Repeated halving method-** The composite sample is spread uniformly over clean surface. It is divided into two equal parts and one part is again thoroughly mixed and the division is repeated to get the required quantity of submitted sample.

## PHYSICAL PURITY

**Physical purity analysis by number-**

**Materials required:** Physical purity work board (diaphanascope), forceps, cups and specimen of objectionable weed seeds

**Diaphanpscope:** It is made up of wooden flat smooth board of 55cm length, 27 cm width and 10 cm height. Reflected light provided from the source at the base covered with opaque glass sheet. A magnifying glass (10 x) is fixed on a stand helping the analyst to separate and observe the seeds from front side.



**Procedure:** The sample is spread uniformly over the physical purity work board

and the following categories of seeds are sorted out with the help of pair of forceps by visual observation and separated in different cups.

1. Other crop seeds
2. Total weed seeds
3. Objectionable weed seeds
4. Objectionable parasite at crop level

**Result:** Reported by number of seeds present in prescribed quantity (g) of submitted sample.

| Number | | | | | | Size of the submitted sample |
|---|---|---|---|---|---|---|
| OCS | | WS | | OWS | | |
| Number | Name | Number | Name | Number | Name | |
| | | | | | | |
| | | | | | | |

**Physical purity analysis by weight**

**Materials required:** physical/precision balance, purity work board, spatula, forceps, cups, seed sample

**Procedure:** The working sample of desired weight is distributed evenly over physical purity work board. Pure seed, other seed and inert matter are separated with the help of spatula and forceps and kept in different cups. No pressure or magnification is applied except for some grasses.

**Calculation:**

Purity (%)= $\frac{weight\ of\ pure\ seed\ (g)}{pure\ seed\ (g)+other\ seed\ (g)+inert\ matterm\ (g)}\ x\ 100$

**Result:** It is presented using one decimal place. Components less than 0.05 % are reported as trace.

| Component | Weight (g) | Remark |
|---|---|---|
| Pure seed | | |

## GERMINABILITY OF SEED LOT

**Paper towel method-** The information pertaining to test sample is written on the mid-portion of the paper towel prior to its proper moistening with distilled water. Moist towel is stretched on a clean table and on the other seed of the paper towel 50 seeds are arranged on its half portion containing 5 rows each of 10 seeds. About 3 cm space is left on lower and right side. Seeds are covered with remaining half portion of the towel and right and lower portion is folded upward to close both the ends. The paper towel is rolled from the right end, wrapped in a wax paper and the ends are tightened with rubber bands. This paper towel is placed vertically in a seed germinator with upward direction of open end.

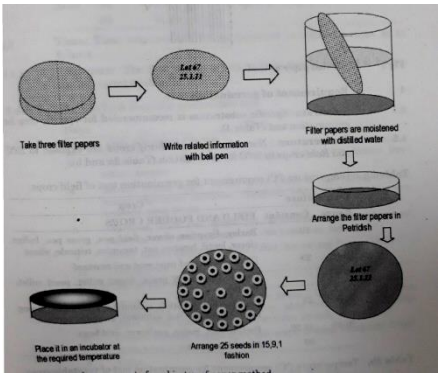
**Sand method-** Sterilized sand is immersed in distilled water. The excess water is removed by keeping the handful of this sand in air and placed in a tray of 6-9 cm depth. Counted seeds are planted on the levelled sand at proper distance and covered with a layer of dry sand. The seeded tray is placed in a germinator.

**Seed germination testing by filter paper method-**

**Materials required**- filter paper, seed germinator, distilled water, Petri dish

**Procedure-**

1. Information related to lot is written on filter paper.
2. Three pieces of circular filter paper including paper with information are moistened with distilled water.
3. The excess moisture is trickled down by placing the moistened filter paper in air.
4. These are placed in Petriplates with the help of pair of forceps. Place 25 seeds on the top of moist filter paper.
5. The closed Petriplate is placed in germinator.




## SEED VIABILITY TESTING

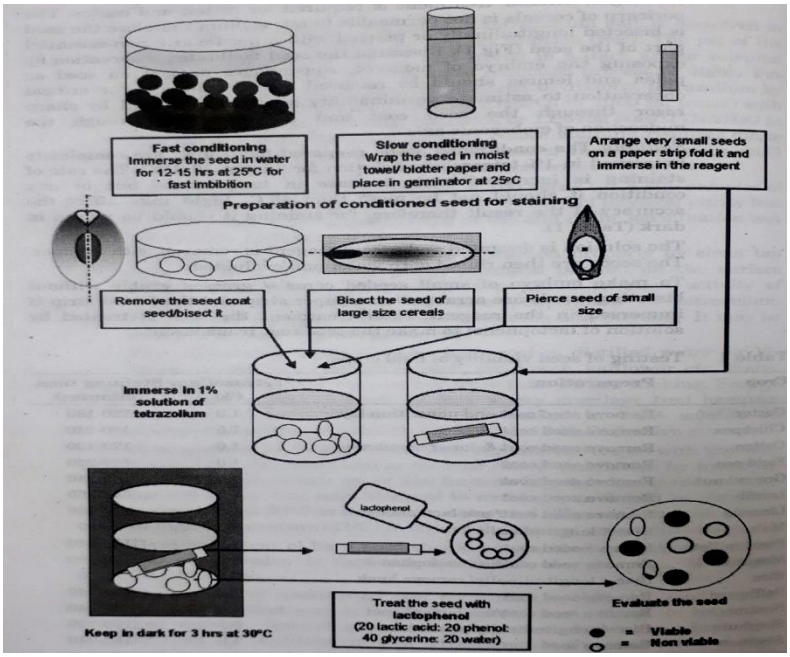
**Principle-** A colourless solution of 2,3,5 triphenyl tetrazolium chloride reacts with hydrogen released by the reduction process in the living cells due to action of enzyme dehydrogenase, which catalyses the reactions in glycolysis and TCA cycle. The malic acid dehydrogenase carries out the reduction of tetrazolium salt in living tissue. It produces a red, stable and non-diffusible substance triphenyl formazan to distinguish from the colourless dead ones.

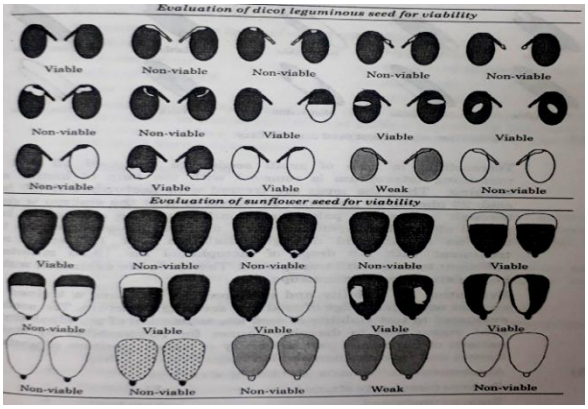
**Reagent:** Tetrazolium salt, water

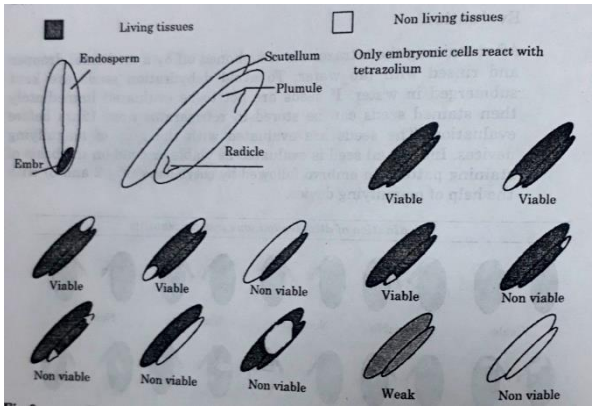
**Equipment:** Blotter, beaker, pH meter, measuring cylinder, electronic balance, razor blades, dissecting knives and needles. Watch glasses, Petridishes, magnifying glass

**Procedure:**

1. One-gram tetrazolium salt is dissolved in 100 ml water to prepare one percent solution. The 1 % solution is used for seeds that are not bisected through the embryo, while 0.1% is used for seeds in which embryo is bisected. pH should be 6.5-7.5.
2. If the pH of water is not in normal range, the buffer solution is prepared as below.
   Solution 1: Dissolve 9.078 g of $KH_2PO_4$ in 1000 ml of water.
   Solution 2: Dissolve 11.876 g of $Na_2HPO_4.2H_2O$ in 1000 ml of water
   Take 400 ml of solution 1 and 600 ml of solution 2 and mix them. In a litre of this buffer solution, Dissolve 10 g of tetrazolium salt.
3. Stored in dark amber coloured bottle.
4. The seed is washed properly by clean tap water.
5. Seeds are fully immersed in distilled water for 12-15 hours.
6. The coat of dicot seed is removed without any damage to cotyledon and embryo. In cereals the pericarp is not permeable to tetrazolium therefore the seed is bisected longitudinally with one clean sliding cut with razor, thus exposing the main structures of embryo.
7. The prepared seed is placed in a suitable container and completely immersed in 1 % tetrazolium solution for 3 hours.



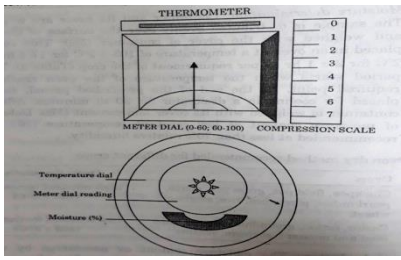




## DETERMINATION OF MOISTURE CONTENT

**Equipment:** Universal moisture meter- It is a machine with Electrical conductivity of moist material is directly proportional to the amount of moisture in it.

I. Compression unit
II. Moisture meter dial
III. Thermometer
IV. Compression knob
V. Cups of different volumes




**Procedure-** A representative sample of prescribed weight and volume is taken and placed in sample cup. It is fixed in lower house of compression unit. Meter is calibrated by pressing "cal" and "bell" with the help of calibration knob. Sample is compressed with the help of compression knob and scale. At required compression, the meter dial (M) is read by pressing the knob "Read" and bell. Temperature (T) is observed by thermometer fixed in between meter dial and compression chamber. The reading M and T are intercepted on the correlator dial by turning the temperature dial. On adjustment of both

the readings, mark of the arrow on the outer reading of temperature dial indicates the moisture percentage.

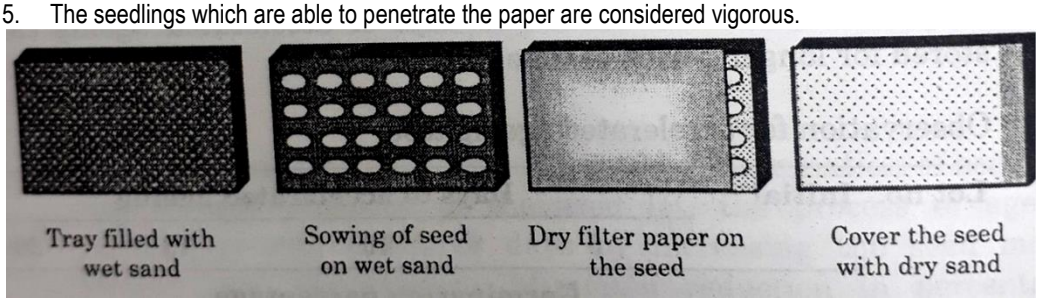
## SEED VIGOUR TESTING

**Paper piercing test-**

**Materials required-** Seeds, tray, filter paper sand etc.

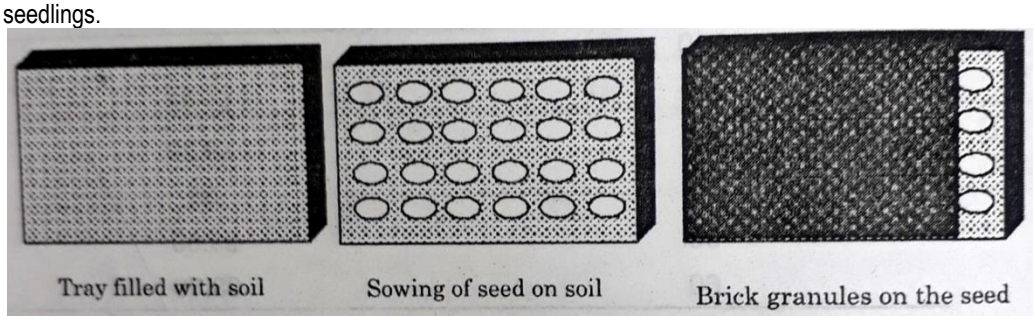
**Procedure-**

1. Seeds are planted on layer of 1.25 cm moist sand.
2. It is covered with especially selected dry filter paper (0.4 mm thickness, 1- bulk, 90g/mt2 m basic weight, 0.3 kg/cm2 dry bursting strength, 1000-5000 m breaking length, 500 ml/min. filtering speed,150 mm wet bursting strength).
3. This filter paper is again covered with 3.00 cm of moist sand.
4. This is kept at 20-25oC for the days required for final count.
5. The seedlings which are able to penetrate the paper are considered vigorous.




**Brick gravel test (Hiltener test)**

1. A tray is filled with soil up-to 3 cm depth.
2. Seeds are placed on the soil surface.
3. After placement, the tray id tightly packed with crushed brick granules of 2-3mm in size at a depth of 3.00 cm.
4. Brick granules put a stress on the emergence and elongation of seedlings and hinder the emergence of weak seedlings.




## GENETIC PURITY DETERMINATION AT SEED LEVEL

**Equipment-** Physical purity work board, spatula, forceps, Petri dishes and notebook.

**Procedure-**

1. The authentic seed sample of the variety under test is examined by visual observation for the expression of morphological traits at seed level.
2. The submitted sample is uniformly spread over the surface of the physical purity work board and observations on the characters are made.
3. Seeds exhibiting differences in distinguishing characters are sorted out and separated on Petri dish.
4. The seeds sorted out as genetically impure are compared with authentic seed for expression of morphological trait.
5. The confirmed genetic impure seeds are counted and reported in number.

## GENETIC PURITY BY GROW OUT TEST

**Sampling-**

**Submitted sample-** The size of submitted sample for grow out test varies along with crop species-

| Crop | Size of sample (g) |
|---|---|
| Genera with seed size similar to pearl-millet | 100 |
| Genera with seeds size similar to beat vulgaris | 250 |
| Sorghum, rice, wheat | 500 |
| Maize, cotton, groundnut soybean | 1000 |

**Working sample-** The size of working sample mainly depends on the test weight and germination percentage of the of the crop to observe the permissible off type plants prescribed as minimum seeds certification standards in the optimum population i.e. minimum 400 plants.

| Maximum permissible off types (%) | Number of plants required |
|---|---|
| 0.10 | 4000 |
| 0.20 | 2000 |
| 0.30 | 1350 |
| 0.50 | 800 |
| 1.00 and above | 400 |

**Land requirements:** In field test is conducted on land which is free form other crops, weeds, and volunteer plants with adequate fertility and irrigation facility.

**Raising of crop-** Standard and recommended cultural practices are adopted for raising of the crop in two replications but the row length, plant to plant, row to row, and plot to plot distances have to be altered as per recommendations. Proper care should be taken to avoid admixture and to maintain optimum plant population. Subsequent thinning and transplanting are not recommended. Authentic sample is grown with same cultural practice at suitable interval.

**Observations:** Each and every plant is examined throughout the growing season with emphasis on the expressions of stable, uniform and distinguishing marker characteristics and time of their expressions. The plants showing deviation are tagged and examined thoroughly to confirm their genetic purity.

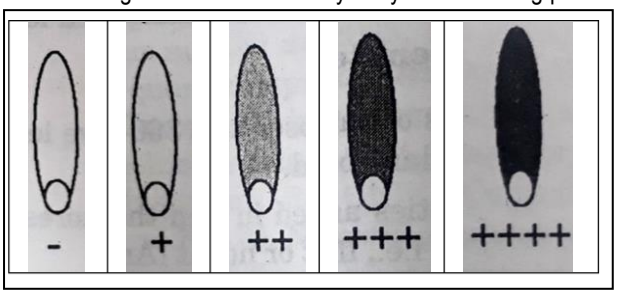
**Calculation:** Percentage of genetic purity is worked out on the basis of number of offtypes and total plant population upto first decimal place. Result is interpreted by using the reject number for prescribed standards with reference to sample size.

| Genetic purity (%) | Reject number for sample size | | | |
|---|---|---|---|---|
| | 100 | 400 | 800 | 2000 |
| 99.5 | 00.5 | 2 | 008 | 010 |
| 99.0 | 01.0 | 8 | 016 | 020 |
| 95.0 | 05.0 | 24 | 048 | 100 |
| 90.0 | 10.0 | 44 | 088 | 200 |
| 85.0 | 15.0 | 64 | 128 | 300 |

## Biochemical tests for genetic purity

**Phenol colour test**

Variety of cereals particularly wheat, rice and sorghum are verified through involvement of enzyme tyrosinase using phenol as a substance.




**Reagents and equipment:** 1% freshly prepared solution of carbolic acid (V/V in water), Petri-plate, beaker, pipette, incubator, filter paper, forceps and distilled water.

**Procedure:**

1. Seeds treated with fungicide are rinsed with methanol prior to soaking.
2. Seeds are placed in a beaker and immersed in distilled water.
3. Two sheets of filter paper are arranged in a Petri plate.
4. Seeds are arranged on filter paper with the help of a pair of forceps keeping the hump portion of the seed upward.
5. 1% solution of carbolic acid is applied in each Petri plate with the help of pipette till ¾ part of the seed is covered.
6. After application of carbolic acid, the Petri plate is covered with lid immediately to avoid the evaporation of carbolic acid.
7. The Petri plate is placed in an incubator for required period.
8. Seeds are observed after prescribed period for development of colour and its intensity to distinguish the genetically impure seeds.
9. Intensity of the colour developed on the seed is observed as no reaction (-); deep olive (+); light brown (++); brown (+++); black (++++)
10. Seeds pure at genetic level expresses similar intensity of colour in response to test.

**KOH bleach test:** Genetic purity of sorghum varieties can be verified on the basis of dark pigmentation present on tagmen of the seed coat of some varieties due to tannic acid.

**Reagents and equipment:** Potassium hydroxide, bleach (NaOCL) and beaker

**Procedure:**

1. 5.25 % of bleach is prepared in water.
2. 1:5 (weight/volume) solution of KOH is prepared with 5.25 % bleach solution
3. Seeds are soaked in this solution for 10 minutes.

4. Seeds are gently swirled in the solution
5. Seeds are placed on filter paper for air drying
6. Varieties are varieties on the basis of dark or light colour developed on the seed.

**Peroxidase activity test:** Presence or absence of peroxidase enzyme in seeds of crops from family of Leguminosae is under genetic control. Therefore, this test is particularly used for DUS testing of soybean.
**Reagent and equipment**: 0.5% (V/V) guaicol, 0.1% (V/V) hydrogen peroxide, razor, test tube and dropper.
Procedure:
1. Coat of 20 seeds sorted as genetically impure is removed with the help of sharp razor.
2. Seed coat is placed in test tube in such a way that one test tube contains coat of only one seed.
3. Guaicol solution of 0.5% is prepared in water.
4. In each test tube 10 drops of 0.5% guaicol solution is added.
5. After 10 minutes, 0.1 % hydrogen peroxide is added in each test tube
6. Development of red colour in the solution shoes presence while no change shows absence of the activity of peroxidase.
7. Seed present with no change in the variety with presence of peroxidase activity or vice versa confirms genetic impurity

## ELECTROPHORETIC METHOD OF GENETIC PURITY TESTING

**Principle:** Changes in coding sequence of proteins result in corresponding replacements in amino acids. They possess ionizable groups and can therefore be made to exist in solution as electrically charged particle. Molecules with higher charge will move faster than those with lower charge. This separation on the basis of size and net electrical charge is electrophoresis.
**Equipment:** gel electrophoresis unit, power supply unit, aspirator, pH meter, mortar pestle, centrifuge, illuminator tray, razor blade
**Preparation of sample:** Seeds are crushed with mortar and pestle and transferred to Eppendorf tube.
**Extraction of proteins:** TRIS hydroxyl methyl amino methane is used and protocol is standardised on the basis of crop. For pearl-millet and soybean 1.21 g of TRIS amino methane is dissolved in 70 ml distilled water. pH is maintained by adding HCL drop wise. The volume is made up 100 ml by distilled water. This extraction medium (0.5 ml) is added in Eppendorf tubes containing seed material and left for 2-3 hours. It is centrifuged at 10oc at 10000-15000 rpm for 10-30 minutes and supernatant is decanted and used for loading.

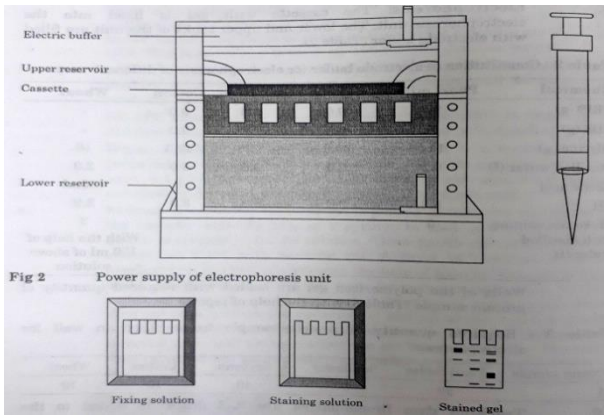


Fig 2 Power supply of electrophoresis unit

**Gel preparation (TRIS HCL buffer):** 22.69 g TRIS for running gel and 7.26 g for staking gel is dissolved in 50 ml distilled water. pH of TRIS HCL buffer for running gel is maintained 8.8 and for staking gel 6.8 with HCL. The final volume is made up-to 100 ml with distilled water.
**Filling of cassette:** Running gel is poured in between the plates of the cassette with the help of syringe in such a way that, no air bubble is trapped in gel solution. ¾ part of cassette is filled with running gel. A layer of distilled water is overlaid on the running gel with the help of pipette. This gel is kept under fluorescent light for one hour for polymerization. Then the water layer is poured off. Stacking gel is poured on running gel and an acrylic comb is placed inside the cassette to make required number of wells. The comb is removed after half an hour when the gel has polymerized.
**Electrophoresis:** The cassette gel is fixed into electrophoresis unit. The lower and upper tanks are filled with electrode buffer. Bromo-phenol dye is added to the electrode buffer in upper tank. Then the unit is connected to power supply.
**Staining:** The staining solutions for different crops are prepared as per the protocols. The staining tray is filled with stain and gel is placed in it. It is incubated till the bands are developed.

## SEED PRODUCTION OF CEREALS

**RICE:** Isolation distance is 3m.
**For hybrid rice-** The field should be isolated from other paddy field including commercial hybrids of same variety, and same hybrid not confirming to varietal purity requirement for certification at least by 200 meters for foundation seed (A, B an dR line production) and by 100 meters for hybrid seed production (A x R).
**Planting ratio:** 2:10-12

**WHEAT:**

Isolation distance - 3m. In case the variety is susceptible to loose-smut, isolation distance of 180 m from seed field and other field of wheat is recommended. The Indian minimum seed certification standards require only 150 m isolation from other

wheat fields wherein loose smut infection is in excess of 0.1% in case of foundation seed production and 0.5 % in case of certified seed production.

**BARLEY**

**Isolation distance-** 3 m In case the variety is susceptible to loose-smut, an isolation distance of 150 m from other wheat fields wherein loose smut infection is in excess of 0.1% in case of foundation seed production and 0.5 % in case of certified seed production is required.
**Minor millets:** Isolation distance - 3 m

**Two steps of Hybrid seed production:**

1. **Maintenance of inbred lines (foundation seed production)**
2. **Hybrid seed production (certified seed production)**

**SORGHUM**

**Open pollinated varieties-**

| Contaminant | Isolation distance (m) | |
|---|---|---|
| | Foundation | Certified |
| Field of other varieties of grain and dual-purpose sorghum and the same variety not confirming to varietal purity requirement for certification | 200 | 100 |
| Johnson grass | 400 | 400 |
| Forage sorghum | 400 | 400 |

**Hybrid seed production-** Planting ratio-4:2

| Contaminant | Isolation distance (m) | |
|---|---|---|
| | Foundation | Certified |
| Field of other varieties of grain and dual-purpose sorghum and the same variety not confirming to varietal purity requirement for certification | 300 | 200 |
| Field of other hybrids having same male parent | 00 | 5 |
| Johnson grass | 400 | 400 |
| Forage sorghum | 400 | 400 |

**PEARL-MILLET**
**Open pollinated varieties-**

| Contaminant | Isolation distance (m) | |
|---|---|---|
| | Foundation | Certified |
| Field of other varieties and the same variety not confirming to varietal purity requirement for certification | 400 | 200 |

**Hybrid seed production-** Planting ratio-4:2

| Contaminant | Isolation distance (m) | |
|---|---|---|
| | Foundation | Certified |
| Field of other varieties and hybrids or same line not confirming to varietal purity requirement for certification | 1000 | 200 |
| Field of other hybrids having same male parent | 00 | 5 |
| Differential blooming to modify isolation is not permitted. | | |

**MAIZE**
**Open pollinated varieties-**

| Contaminant | Isolation distance (m) | |
|---|---|---|
| | Foundation | Certified |
| Field of other varieties and the same variety not confirming to varietal purity requirement for certification | 400 | 200 |

**Hybrid seed production-**

| Contaminant | Isolation distance (m) | |
|---|---|---|
| | Foundation | Certified |
| Field of any maize with same kernel colour and texture | 400 | 200 |
| Maize with different kernel colour and texture | 600 m | 300 m |

| | | |
|---|---|---|
| Same variety not confirming to varietal purity requirement for certification | 400 m | 200 m |
| Other hybrids having common male parent | 00 | 5 |
| Differential blooming to modify isolation is permitted provided up-to 5 % plants do not have receptive silks. | | |

**Seed production of pulses**

**Minimum isolation distance**

| Crop | Isolation distance (m) | |
|---|---|---|
| | Foundation seed | Certified seed |
| Red gram | 200 | 100 |
| Gram | 10 | 5 |
| Pea | 10 | 5 |
| Black gram and green gram | 10 | 5 |
| Lentil | 10 | 5 |
| Cow pea | 10 | 5 |

**Seed production of Oilseed crops**

| Crop | Isolation distance (m) | |
|---|---|---|
| | Foundation seed | Certified seed |
| Groundnut | 3m | |
| Rapeseed and mustard (SI) | 100 | 50 |
| Rapeseed and mustard (SC) | 50 | 25 |
| Linseed | 50 | 25 |
| Sesame | 100 | 50 |
| Castor | 300 | 150 |

**HYBRID SEED PRODUCTION IN SUNFLOWER**

**Maintenance of parental lines-**

Planting ratio-3:1

Isolation- Seed fields from other sunflower lines not confirming to varietal purity requirement for certification-600 m.

**Production of hybrid seed-**

Planting ratio-3:1

Isolation- Seed fields from other sunflower varieties, commercial hybrid of same variety and field of same hybrid seed production not confirming to varietal purity requirement for certification- 400m

**HYBRID SEED PRODUCTION IN COTTON**

**Isolation requirement**- 50 meters for foundation seed class and 30 meters for certified seed production form fields of other varieties of same species, fields of same variety not confirming to varietal purity requirement for certification, and 5 meters from varieties of different species (different ploidy levels).

**SEED PRODUCTION IN BRINJAL AND TOMATO**

**Minimum isolation distance**

| Contaminator | Isolation distance (m) | | | |
|---|---|---|---|---|
| | Foundation | | Certified | |
| | Brinjal | Tomato | Brinjal | Tomato |
| Other varieties | 200 | 200 | 200 | 100 |
| Seed production of same hybrid without confirming seed certification standards | 200 | 200 | 200 | 100 |
| Between blocks of parental lines | | | 5 | |