Practical Manual

Principles of Seed Technology

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PRACTICAL NO.1

Object; Seed Production in Major Cereal Crops.

Wheat

(1) Selection of seed plot: The plot to be used for seed production of wheat shall be with deep ploughing, followed by harrowing and leveling. Pre-sowing irrigation should be given for free from weeds and volunteer plants. The plot should be well drained. Prepare the land uniform germination.

(2) Isolation distance: Wheat is normally a self-pollinated crop, however, natural crosspollination to the extent of 1 to 4 percent occurs. So an isolation distance of 3 meter should be kept in all the side of seed plot to avoid natural crossing. If a variety of the seed plot is likely to get infected by loose smut then isolation distance of 180 meters between seed field and other field of wheat is recommended.

(3) Planting time and seed rate:

1) Long duration (late maturing) varieties may be sown during the first fortnight of November.

2) Short (early) and medium duration varieties may be sown during second fortnight of November.

3) The seed crop should be sown in rows at spacing of 20 to 22.5 cm to a depth of 5 cm. 4) The recommended seed rate for seed crop is 85 to 100 kg /ha.

(4) Cultural practices

1) Fertilizer: The recommended doses of fertilizer is 80 kg nitrogen, 60 kg phosphorous and 40 kg potash per hectare. Apply all the quantity of phosphorous and potash fertilizers at the time of sowing while 50 % quantity of nitrogen at time of sowing and remaining 50 % nitrogen at the crown root initiation stage i.e. 30 to 35 days after sowing**.**

2) Irrigation: Depending upon the soil texture and structure about 4 to 6 irrigations are sufficient. 1st irrigation at 30 to 35 days after sowing and other irrigations at late tillering, panicle emergence, flowering, milk and dough stages should be given

3**) Inter-culturing and weeding**: Periodically inter culturing and weeding should be

carried out to keep the crop free from weeds. Chemical weedicides like 2, 4-D @ 0.5 kg a.i. per hectare and pendimethalin @ 1 kg a.i. per hectare in 750 litres of water should also be used for effective control of weeds.

4) Plant protection:

I. For the control of termites, use chlorpyriphos 20 EC @ 2.3 litres/ha with irrigation water.

II. For control of stem borer apply carbofuran 3 G @ 25 kg/ha two weeks after germination or spray the crop with endosulfan 35 EC @ 1.5 litres per hectare. III. Seed treatment

(5) Roguing: Two to three roguing are sufficient

1) 1 st roguing may be done at the time of heading to remove off types and plants infected with loose smut.with late flowering based upon ear head (panicle) characters.

3) 3 rd roguing should be done at the time of maturity based upon variation in ear head colour. Colour of awns and ear head types as well as volunteer plants and weed plants should be removed**.**

Harvesting and threshing: Harvesting may be done by sickle and threshing with thresher. Care should be taken to avoid mechanical mixture.

(1) Processing: Wheat seeds should have 9 to 10 percent moisture content for storing purpose. To maintain good quality of seeds, it should be cleaned, treated with fungicide and should be properly bagged. The seed should be stored in a dry, clean and rodent proof warehouse.

(2) Yield: The average seed yield should be between 40 to 45 quintals per hectare.

Minimum seed certification standard

	Foundation seed	**Certified seed**
Germination (%)	**85**	**85**
Genetic purity (%)	**98**	**98**
Inert matter (%)	**2**	**2**
Other crop seeds (No./kg)	**10**	**10**
Weed seeds (No./kg)	**10**	**10**
Diseased seeds (%)	**0.05**	**0.25**
Moisture (%)	**10**	**10**

Rice (Paddy)

1. Selection of seed plot: The plot should be free from weeds and volunteer plants and should have not been used for growing the same crop in previous year or season. Prepare the land with deep ploughing followed by harrowing so that the transplanted seedlings establish quickly. A plot should be kept flooded for a week or ten days before transplanting.

2. Isolation chamber: The extent of cross pollination in rice varies from 0 to 6.8 % hence it is necessary to keep the plot isolated at least by 3 meters from other rice plot for pure seed production.

3. Cultural practices: The paddy crop must be grown by direct sowing or by transplanting. For seed production transplanting method is desirable.

I. Raising nursery: Land selected for paddy nursery should not have paddy as previous crop to avoid varietal mixture due to volunteer plant. The appropriate time of sowing nursery for early duration varieties is from 10th to 25th June and for late duration varieties, it is 25th May to 10th June. Long and narrow nursery beds (1 m x 10 m) are more ideal. Prepare raise bed to facilitate drainage of excess water and also to irrigate the nursery uniformly. About 80 to 90 beds of the size 10 m x 3 m are sufficient for raising seedlings to transplant one hectare of land.

II. Seed rate: 20 -25 kg for fine grain varieties, 30-35 kg for coarse grain varieties Seed should be obtained from the source approved by the seed certification agency. The sowing of seeds in nursery may be carried out in row (line) sowing or broadcasting may be done. Irrigate the nursery after sowing the seeds. Recommended plant protection measures and fertilizer application may be made to raise the seedlings successfully. Keep the nursery free of weeds.

III. Uprooting of seedlings and transplanting: Seedling are ready for transplanting after 3 to 4 weeks of sowing. Fertilizer may be applied based on soil test, however, the fertilizer recommendation is 120-60-00 kg N: P: K for later varieties and 100-50-00 kg N: P: K for early and mid-late varieties. Apply whole amount of phosphorus and potash as basal dose at the time of puddling. 50 % nitrogen may be applied as basal dose while 25 % of nitrogen at tillering stage and 25 % at panicle initiation stage. If the land is deficient in zinc, apply

15 kg zinc sulphate per hectare at puddling stage. Spacing should be kept at 20 cm x 15 cm. Maintain a water level of 2.5 to 5 cm of water till milking stage. Drain excess water when the crop does reach to physiological maturity.

V. Plant protection : Stem borer, brown plant hopper, leaf roller and Gundhi bug are the major pests of paddy. a) For the control of stem borer use carbofuran 3 % granules @ 20 kg/ha or phorate 10 % granules @ 10 kg/ha.

b) For the control of brown plant hopper and leaf roller spray endosulphan 35 EC @ 1 litre per hectare

c) Disease : Blast, bacterial leaf blight, bacterial leaf streak and brown spot are the important disease of paddy.

i. For control of blast Hinosan 625 ml per hectare in 625 litres of water one or two times before panicle emergence and once after panicle emergence.

ii. For the control of bacterial leaf blight spray 75 g agrymycine + 500 g copper oxychloride in 500 litres of water per hectare 3 to 4 times at an interval of 10 to 15 days.

iii. For control of bacteria leaf streak spray 12 g of streptocycline or 75 g agromycin in 50 litres of water per hectare at an interval of 10 to 15 days.

iv. For the control of brown spot spray 0.25 percent dithane M-45 or Zineb after 6 weeks of transplanting at an interval of 10 to 12 days. : Keep the crop free from weeds by hand weeding or using chemical herbicides. Butachlor or benthiocarb @ 1.5 kg a.i./ha 5 to 7 days after transplanting.

5. Harvesting and threshing: It is important to harvest the crop when the seed is ripe. The moisture content at this stage varies between 17 to 23 percent. Harvest the crop by sickle or combined harvester. Allow the crop to dry for two to three days till the moisture content reduce to 12 to 13 percent. Clean the seeds to remove chalf, durt, empty husks and light seeds by winnowing. Store in a gunny bags in a cool and dry place on wooden racks.

The average paddy seed yield should be from 50 to 60 quintals per hectare depending upon the varieties.

Minimum Seed Certification Standard

	Foundation seed	Certified seed
Germination (%)	**80**	**80**
Genetic purity (%)	**98**	**98**
Inert matter (%)	**2**	**2**
Other crop seeds (No./kg)	**10**	**10**
Weed seeds (No./kg)	**10**	**10**
Diseased seeds (%)	**0.01**	**0.05**
Moisture (%)	**13**	**13**

PRACTICAL NO.2

Object; Seed Production in Pulse Crops.

PIGEONPEA / REDGRAM

➢ Pigeon pea / Red gram is an important pulse crop next to chickpea in India.

➢ The major pigeon pea growing countries are India, Uganda, Kenya, West Indies, Puerto Rico, Dominican Republic in the Caribbean region and Burma.

➢ India is a largest producer i.e. 90% of the world's production. It is grown in kharif season.

➢ In India it is mainly cultivated in Gujarat, Maharashtra, Madhya Pradesh, Uttar Pradesh, Karnataka and Andhra Pradesh.

1. Name of Crop; Pigeonpea, Redgram, Arhar(Hindi), Toor (Gujarati)

2**. Botanical Name**; Cajanus cajan L.

3. Family: Fabaceae

4. Chromosome No. : 2n = 22

5. Center of Origin: India

6. Mode of pollination: Often cross pollination

7. Cross pollination %: 5 - 48 % (mostly by insects)

Seed Production of Pigeon pea

(1) Selection of seed plot: The plot to be used for seed production of pigeon pea shall be free from weeds and volunteer plants. The plot should be well drained. Prepare the land with deep ploughing, followed by harrowing and leveling.

(2) Isolation distance: Pigeon pea is normally an often cross-pollinated crop, however, natural cross-pollination to the extent of 65 percent occurs. So an isolation distance of 200 meter should be kept for foundation seed class and 100 meter for certified class side of seed plot to avoid natural crossing.

(3) Planting time and seed rate: Sowing of seed crop in first week of June is

recommended for obtaining higher yields. Seed crop should be sown in rows at spacing of Row to Row- 60 to 75 cm

Plant to plant- 25 to 30 cm a depth of 5 cm.

The recommended seed rate for seed crop is 12 to 15 kg /ha.

(4) Cultural practices: (a) Fertilizer: The recommended doses of fertilizer is 25 kg nitrogen, 50 kg phosphorous per hectare. All the quantity of nitrogen and phosphorous should apply in drilled at time of sowing of seed.

(b) Irrigation: one to two light irrigation prior to onset of monsoons may be necessary.if rain disturibution is irregular and weather remains dry for prolonged periods, one irrigation at flowering time and subsequent irrigation after flowering are necessary.

(c) Inter-culturing and weeding: Periodically inter culturing and weeding should be carried out to keep the crop free from weeds. Chemical weedicides like 2,4-D @ 0.5 kg a.i. per hectare and pendimethalin @ 1 kg a.i. per hectare in 750 litres of water should also be used for effective control of weeds.

(d) Plant protection: For the control of pod fly spray endosulphan 1.25 liters per hectare or monocrotophos at 750 ml per hectare in 250 liters of water. For the control of pod bug or plume moth and gram catter piller, spray, 750 ml of monocrotophos or dust 25 kg Malathion 5 % dust per hectare.

(5) Roguing: rogue the off-type plants and diseased plants affected by wilt, leaf spot and stem canker, YVMV and sterility virus from seed field from time to time as required.

(**6) Harvesting and threshing:** Harvesting may be done by sickle and threshing with sticks. Care should be taken to avoid mechanical mixture.

(7) Processing: Pigeon pea seeds should have 8 to 10 percent moisture content for storing purpose. To maintain good quality of seeds, it should be cleaned, treated with fungicide and should be properly bagged. The seed should be stored in a dry, clean and rodent proof warehouse.

(8) Yield: The average seed yield should be between 20 to 25 qtls per hectare.

Minimum seed certification standard

	Foundation seed	**certified seed**
Germination (%)	**85**	**85**
Genetic purity (%)	**98**	**98**

Inert matter (%)	**2**	**2**
Other crop seeds (No. /kg)	**10**	**10**
Weed seeds (No. /kg)	**10**	**10**
Diseased seeds (%)	**0.05**	**0.25**
Moisture (%)	**9**	**9**

BLACK GRAM AND GREEN GRAM

1) Selection of seed plot: The plot to be used for seed production shall be free from weeds and volunteer plants. The plot should be well drained.

2) Isolation distance: Since the pollen shedding takes place long before the petals open. Self-pollination is the rule. Therefore, an isolation of ten meters for foundation seed and five meters for certified seed class from fields of other variety not Conforming to varietal purity requirements for certification is necessary.

3) Planting time and seed rate:

The crop comes up well in the kharif season. However, it can be sown either in the second week of February.

Spacing (Kharif and Rabi)

Row to Row- 30 to 45 cm

Plant to plant- 7 to 10 cm

(**Spring and summer**)

Row to Row- 20 to 25 cm

Plant to plant- 7 to 10 cm

The recommended **seed rate** for Kharif and Rabi seed crop is 15 to 20 kg /ha.

The recommended **seed rate** for spring and summer seed crop is 25 to 30 kg /ha.

4) Cultural practices:

(e) Fertilizer: The recommended doses of fertilizer is 20 kg nitrogen, 35to 40 kg phosphorous per hectare. All the quantity of nitrogen and phosphorous should apply in drilled at time of sowing of seed.
(f) Irrigation: Frequent irrigation for spring and summer may be necessary. The kharif crop does not required irrigation.

(**g) Inter-culturing and weeding:** Periodically inter culturing and weeding should be carried out to keep the crop free from weeds. Spraying chemical weedicides like Treflan @ 1.0 kg a.i. per 1000 litre of water per hectare.

(h) Plant protection :
For the control of aphids and white fly spray Metasystox 625 ml per hectare in 625 liter of water and imidacloprid 3.0 ml/10 lit. water.
For the control of pod boror and catter pillars spray, 3 ml of corozon in 10 liter of water.

Roguing : Rogue the off-type plants and diseased plants from seed field from time to time as required.

5) Harvesting and threshing : Harvesting may be done when the seeds are fully Matured and pods turn black. Threshing can be done by hand to avoid injury to seeds. Care should be taken to avoid mechanical mixture.

6) Processing : Seeds should have 8 to 10 percent moisture content for storing purpose. To maintain good quality of seeds, it should be cleaned, treated with fungicide and should be properly bagged. The seed should be stored in a dry, clean and rodent proof warehouse.

7) Yield : The average seed yield should be between 8 to 10 qtls /ha. (Green gram)
10 to 15 qtls /ha. (Black gram)

PRACTICAL– 3

Object; Seed Production in Major Oil seed Crops.

GROUNDNUT

1.Selection of seed plot: The plot to be used for seed production shall be free from weeds and volunteer plants. The plot should be well drained and preferably sandy loam rich in humus content.

2.Isolation distance: Groundnut is a completely self fertilized crop. The percentage of natural crossing is practically negligible. Cross pollination does not take place because the stigma remains enclosed in the keel even in fully opened flowers. Therefore, an isolation of three meters from fields of other variety.

3.Planting time and seed rate :
The crop comes up well in the kharif season. Mid June to first week of July.
Spacing
(for Spreading varieties) Row to Row- 45 to 60 cm
Plant to plant- 10 to 15 cm

(For Bunchy varieties)

Row to Row- 30 cm
Plant to plant- 10 to 15 cm

The recommended seed rate for Spreading varieties 60 to 80 kg /ha.
The recommended seed rate for Bunchy varieties 80 to 100 kg /ha.

4.Cultural practices :

(i) Fertilizer: The recommended doses of fertilizer are 20 kg nitrogen, 50 to 80 kg phosphorous and 30 to 40 kg potash per hectare. All the quantity of nitrogen and phosphorous should apply in drilled at time of sowing of seed.

(j) Irrigation: The kharif crop does not required irrigation. However, in situations of prolonged drought one or two irrigation may be necessary

(k) Inter-culturing and weeding: Periodically inter culturing and weeding should be carried out to keep the crop free from weeds. Spraying chemical weedicides like prometrin, lasso (1 to 2 kg active ingredient dissolved in 500 to 600 lit. of water per hectare) immediately after sowing has been found useful.

(l) Plant protection :
For the control of hairy catter piller spray 3 ml of corozon or proclem in 10 liter of water. For aphids Metasystox 625 ml per hectare in 625 liter of water and imidacloprid 3.0 ml/10 lit. water. For the control of grub apply carbofuran 10 % granules (12 kg per ha.) at the time of sowing.
Roguing : Rogue the off-type plants on the basis of plant size, colour of leaflets, flower colour and diseased plants from seed field from time to time as required.
5.Harvesting and threshing : Harvesting may be done when leaves start yellowing and fall down. At this stage the pods become reticulated and within it the seed is separated from the shell of the pod. Threshing can be done by hand to avoid injury to seeds. Care should be taken to avoid mechanical mixture.
6.Processing : Seeds should have 8 to 9 percent moisture content for storing purpose. To maintain good quality of seeds, it should be cleaned, treated with fungicide and should be properly bagged. The seed should be stored in a cool dry, clean and rodent proof warehouse.
7. Yield : The average seed yield should be between 15 to 20 qtls /ha

SEED PRODUCTION IN MAJOR OILSEEDS: SOYABEAN, RAPESEED & MUSTARD

		Soyabean	Mustard /Rapeseed
1.	Land Requirement	Medium to black, well drained, previous season soyabean grown land should be avoided.	Medium to black, well drained, previous season mustard crop grown land should be avoided.
2.	Spacing (cm)	45 × 10	45 × 15
3.	Fertilizer NPK (kg/ha)	50:75:00	50:25:00
4.	Seed Rate (kg/ha)	75 kg/ha	5 kg/ha
5.	Isolation distance in meter	3 (all stages)	50 (Foundation) 25 (Certified)
6.	Germination %	70%	70%
7.	Inspections	2/3	2/3
8.	Roughing	Off types, diseased plant should be removed as when noticed.	Off types plants should be roughed out before anthesis.

PRACTICAL– 4

Object; Seed Production in Vegetable Crops

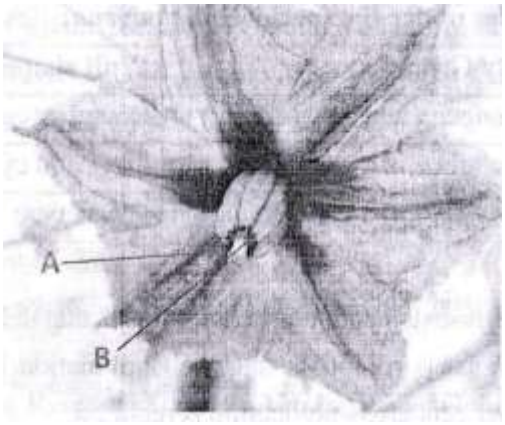
BREEDING VEGETABLE CROPS:BRINJAL

The brinjal also knows as "Egg plant" is a member of the nightshade family, along with the tomato, pepper and potato. Brinjal is grown commonly in almost all the parts of the country and liked by both poor and rich. It is rich in Vitamin A and B.

Name of crop; Brinjal, Egg plant, Guinea squash
Botanical Name; Solanum melongena L.
Family; Solanaceae
Chromosome Number; 2n = 24
Center of Origin; Central Asia
Mode of pollination; Often cross pollination

Four types of flower are found depending upon the length of the style:
1. Long style with big sized ovary.
2. Medium styled with medium rudimentary ovary, which do not producing fruits.
3. Pseudo- short styled with rudimentary ovary, which do not producing fruits.
4. True short styled with very rudimentary ovary, which do not producing fruits.
5 Stigma receptivity is highest during anthesis i.g. flower opening. The receptivity of stigma can be observed from its plump and shiny appearance which gradually becomes brown with the loss of receptivity.
6 Stigma becomes receptive until about four days to flower opening.

Brinjal flower A. Stigma B. Anther cone



Seed Production in Brinjal, Chilli and Tomato:

Nucleus, breeder and foundation seeds are produced by selfing in brinjal, chilli and tomato. Hybrids seed is produced by hand emasculation and hand pollination. Anthersform cone around bilobed stigma. In chilli, CGMS system is utilized to produce hybrid where, as usual, A line is maintained by B line (maintainer, non-restorer line), B line and R line (restorer) by selfing and hybrid seed is produced by crossing A line with R line.

During the certified seed production, isolation distance remains 200 m for foundation seeds and 100-200 m for certified seeds. Though these are self pollinated crops, some aount of cross pollination occurs by insects. Inspections are made during vegetative, flowering and maturity stages

	Tomato		Brinjal	
	Foundation	Certified	Foundation	Certified

Isolation distance (m)	200	200	200	100
Germination (%)	70	70	70	70
Genetic purity (%)	98	98	98	98
Inert matter (%)	2	2	2	2
Other crop seeds (No./kg)	None	None	5	10
Weed seeds (No./kg)	None	None	None	None
Moisture (%)	8	8	8	8

Cucurbits (bottle gourd and ridge gourd)

Bottle gourd and ridge gourd are monoecious crops (separate male flower and female flowers on same plant) and so higamount of cross pollination occurs. Female flowers can bevisualized on plant as ovary with rudimentary anthers (pistillate) and in male flowers ovary isabsent (staminate) but 3 anthers are present. To prevent selfing male flowers are plucked off at bud stage and hand pollination is done for crossing. Isolation distance is 1500m for foundation seeds and 1000m for certified seeds. Four inspections are made, 1st during vegetative stage, 2nd and 3rd during flowering stage and 4th during maturity stage.

	Foundation seed	Certified Seed
Isolation distance (m)	1500	1000
Germination (%)	60	60
Genetic purity (%)	98	98
Other crop seeds (No./kg)	5	10

Weed seeds (No./kg)	None	None
Objectionable weed seeds (No./kg)	None	None
Moisture (%)	7	7

PRACTICAL NO.5

OBJECT; PRINCIPLES AND PROCEDURES OF SEED SAMPLING AND PHYSICAL PURITY TEST

What is Seed Sampling?

The process of taking out a representative seed sample of suitable size from a seed lot forvarious tests is known seed sampling.

Objectives:

1.Sampling is done to get a uniform and representative sample from a seed lot. The sizeof the submitted sample required for testing is small as compared to the size of the lot,

therefore, care must be taken to ensure that the submitted sample represents the lot of the seed to be tested.

2.Hence it is essential that the samples be prepared in accordance to ISTA rules to ensure that the small size sample should represent truly and in the same proportion all constituents of seed lot.

2. Principles for sampling the seed lot:

1.Before sampling of a seed lot is carried out, the sample should be satisfied that the seed lots do not show any heterogeneity.

2.The size of the seed lot shall also not exceed certain limits e.g. small seeded crops, the seed lot shall not exceed 10,000 kg, for the large seeded crops 20,000 kg and for maize 40,000 kg is permitted limits.

3.The seed lot shall be in bags or other containers which should be sealed and labeled for identification by a single lot designation. In case of loose seed which can not be sealed, international seed lot certificate can be refused.

4.At the time of sampling, all containers must be labeled to show a lot identification corresponding to the lot identification of the certificate

Methods of sampling

1.Hand sampling

This is followed for sampling the non free flowing seeds or chaffy and fuzzy seeds such as cotton, tomato, grass seeds etc. In this method, it is very difficult to take samples from the deeper layers of bag. To over come this, bags are emptied completely or partly and then seed samples are taken. While removing the samples from the containers, care should be taken to close the fingers tightly so that no seeds escape.

2.Sampling with triers/Probe

By using appropriate triers, samples can be taken from bags or from bulk. The triers are used for taking free flowing seed samples.

Bin samplers

Used for drawing samples from the lots stored in the bins.

Nobbe Trier

The name was given after the father of seed testing Fredrick Nobbe. This trier is madein different dimensions to suit various kinds of seeds. It has a pointed

tube long enough to reach the centre of the bag with an oval slot near the pointed end. The length is very small. This is suitable for sampling seeds in bag not in bulk.

Sleeve type triers or stick triers

It is the most commonly used trier for sampling: There are two types *viz.*, 1.With compartments 2. Without compartments. It consists of a hollow brass tube inside with a closely fitting outer sleeve or jacket which has a solid pointed end. Both the inner tube as well as the outer tube have been provided with openings or slots on their walls. When the inner tube is turned, the slots in the tube and the sleeve are in line. The inner tube may or may not have partitions.

This trier may be used horizontally or vertically. This is diagonally inserted at anangle of 30°C in the closed position till it reaches the centre of the bag. Then the slots are opened by giving a half turn in clockwise direction and gently agitated with inward push and jerk, so that the seeds will fill each compartment through the openings from different layersof the bag, then it is again closed and with drawn and emptied in a plastic bucket

Method of preparing composite samples

1.When the primary samples appear uniform they are combined and thoroughly mixed to form the composite sample.

2.From composite sample, submitted sample of requisite weight or more is obtained either by repeated halving or by abstracting and subsequently combining small random portions.

Types of sampling

Primary sample

Each probe or handful of sample taken either in bag or in bulk is called primary sample.

Composite sample

All the primary samples drawn are combined together in suitable container to

form a composite sample.

3.Submitted sample

When the composite sample is properly reduced to the required size that to be submitted to the seed testing laboratory, it is called submitted sample. Submitted sample of requisite weight or more is obtained by repeated halving or by abstracting and subsequently combining small random portions.

4.Working sample

It is the reduced sample with required weight obtained from the submitted sample after repeated mixing and dividing with which the seed quality tests are conducted in seed testing laboratory.

	
Trier	**Trier**

 Sampling Clover Seed	
Sampling Process	**Sample divider**
Fig: 6.2 Seed Sampling Apparatus	

Procedure for sampling the seed lot:

1.Using the sampler vertically or horizontally, it should be inserted diagonally out the bagor container.

2.seed in bulk, vertical insertion is more practicable.

3.The trier is thrust into the bag in closed position, then opened and turned a couple oftimes or gently agitated to fill it completely.

4.Afterwards, it is turned to close again, withdrawn and emptied into a suitable container.

5.The seed should not be damaged while closing the trier.

6.The stick trier upto a certain diameter can be used through the walls of coarsely wovenjute or other similar materials.

Note: The samples meant for germination test should not be packed in moisture proof containers. A separate sample for moisture test should be packed in a moisture proof container.

Preparation of submitted sample:

If primary samples appear uniform, they all shall be combined and mixed to form the composite sample. From that the submitted sample is obtained by one of the laboratory methods described below for preparing working samples. If the composite sample is of appropriate size, it may be regarded as the submitted samples without reduction. The submitted sample of prescribed weight must be packed in clean cotton or jute bags, or very long; paper bags along with the slip.

Sampling Intensity:

For seed lost in bags or other containers of similar capacity, the following sampling intensity is regarded as the minimum requirement:

Sr. No.	**Seed containers/ Bulk heaps**	**No. of primary samples**

	When seeds are in containers	
1.	Upto 5 containers	One primary sample from each container and take at least five primary samples.
2.	6-30 containers	One primary sample from every three containers, but total number of primary samples must not beless than five.
3.	31-400 containers	One primer sample from every five or ten containers, but total number of primary samples must not be less than ten.
4.	401 or morecontainers	One primer sample from every seven or takesamples from 80 containers, but total number of primary samples must not be less than ten.
	When seeds are in bags or heaps	
5.	Upto 1500 kg	At least 5 primary samples, except that lost lessthan 50 kg fewer but not less than 3 samples need to be taken.
6.	1501 to 3000 kg	One primary sample for each 300 kg, but not less than 10.
7.	3001 to 20,000 kg	One primary sample for each 500 kg, but not less than 10.
8.	20,001 & above	One primary sample for each 700 kg, but not less than 10.

Methods for preparing a working sample:

One of the following methods is used to prepare working sample.

Mechanical method:

There are four different types of mechanical divider.

(a) Conical divider

(b) Soil type divider

(c) Centrifugal divider

(d) Precision divider

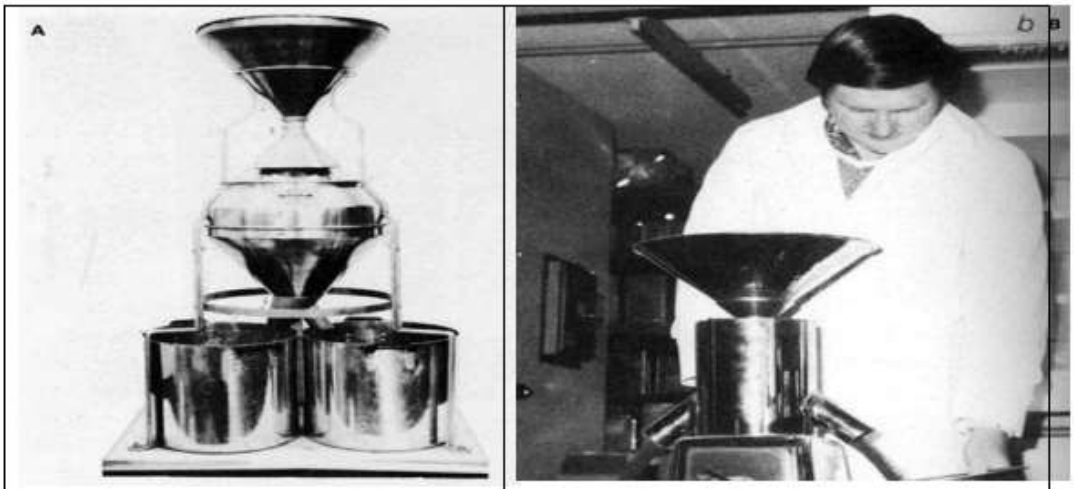


Fig. 6.3 Seed dividers (A) Boerner (B) Gamet.

Random cup method:

In this method, 6-8 small cups are placed on a tray at random. After preliminary mixing, the seed is poured uniformly over the tray and the seed that falls in to the cup is taken as a working sample. For a particular group of similar species, a certain size of cup is needed. At least six cups should be taken. If minimum weight is not obtained, one or more cups can also be added.

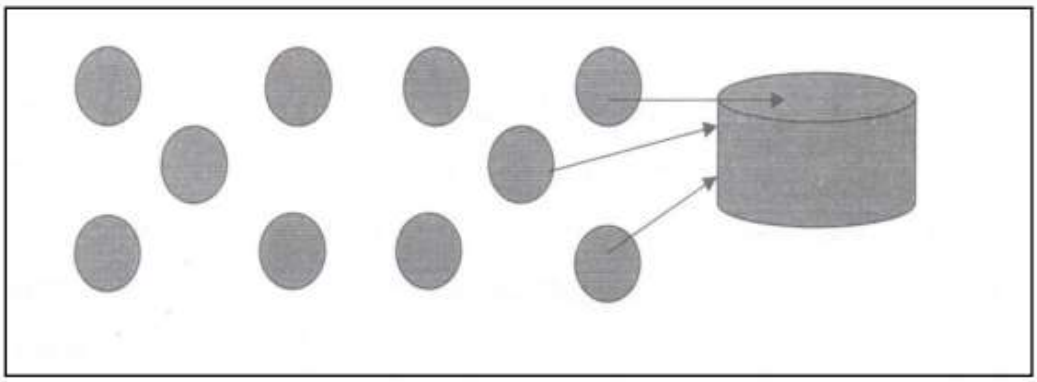


Fig. 6.4 : Preparation of working sample by random cup method

Modified halving method:

In this method, an apparatus comprises of a tray in which a grid of equal sized cubicalcells are fitted, open at the top and every alternate one having no bottom. After preliminary mixing, the seed is poured evenly over the grid. When the grid is lifted, approximately half the sample remains on the tray. This process can be repeated until required sample size is obtained.

Spoon method:

This method is only permitted for small seeded species. A tray, a spatula and a spoon with a straight front edge are required. After preliminary mixing, the seed is poured evenly over the tray. The tray must not be shaken while taking samples. Take the spoon in one hand and the spatula in the other hand and using both, small portions of seed are drawn from more than three random places. Sufficient quantity of seed is taken to constitute the working sample.

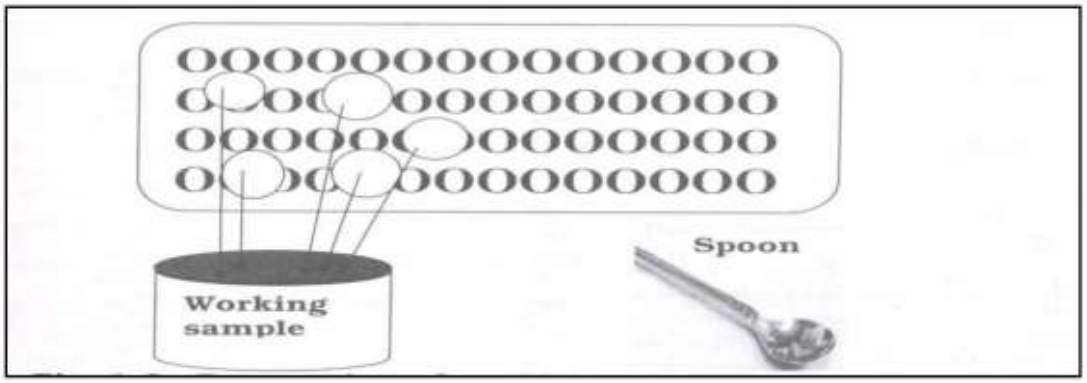


Fig. 6.6: Preparation of working sample by spoon method

Combine method:

It comprises the benefits of rapid reduction in sample size from mechanical method and accuracy of the spoon method. Once this method is established in a laboratory, it very efficient.

Table 6.1: Crop-wise submitted and working sample size for various tests.

Crop	Size of seed lot (Kg)	Size of submitted sample (g)	Size of working Sample for purity analysis(g)	Sample count of other species(g)
Paddy	20,000	400	40	400
Wheat	20,000	1000	120	1000
Maize	40,000	1000	900	1000
Sorghum	10,000	900	90	900
Bajra	10,000	150	15	150
Redgram	20,000	1000	300	1000
Greengram	20,000	1000	120	1000
Blackgram	20,000	1000	150	1000
Bengalgram	20,000	1000	1000	1000
Cowpea	20,000	1000	400	1000
Soybean	20,000	1000	500	1000
Groundnut(pods)	20,000	1000	1000	1000
Groundnut(Kernels)	20,000	1000	600	1000
Gingelly	10,000	70	7	70

Sunflower(variety)	20,000	1000	250	1000
Sunflower(Hybrid)	20,000	1000	125	250
Cotton linted(variety)	20,000	1000	350	1000
Cotton delinted(variety)	20,000	350	35	350
Cotton linted(hybrid)	20,000	350	35	350
Cotton delinted (hybrid)	20,000	250	25	250
Brinjal	10,000	150	15	150
Chillies	10,000	150	15	150
Bhendi	10,000	150	15	150
Tomato(variety)	10,000	70	7	70
Tomato(hybrid)	10,000	7	7	7
Cabbage	10,000	100	10	100
Cauliflower	10,000	100	10	100
Knolkhol	10,000	100	10	100

PHYSICAL PURITY ANALYSIS OF SEEDS

What is Physical Purity?

Physical purity analysis tells us the proportion of pure seed component in the seed lot as well as the proportion of other crop seed, weed seed and inert matter by weight in percentage for which Seed Standards have been prescribed. Thus, it helps in:

1. Improving the plant stand (by increasing the pure seed component).
2. Raising a pure crop (by eliminating other crop seed and weed seeds).
3. Raising a disease free-crop (by eliminating inert matter).
4. In the use of seed drill (by selecting uniform particles).

There is a need for physical purity analysis for:

a) Seed Certification or Seed Law Enforcement Agencies to judge that the seed lot conforms to the prescribed standards.

b) Seed processing plants for using right kind of processing equipment.

c) Physical purity analysis is a pre-requisite for germination test because 'pure seed' component is used for germination testing.

Objectives:

1. To determine the composition by weight of the sample being tested and by inference the composition of the seed lot.

2. To determine the identity of various species of seed and inert matter particles constituting the sample. [Purity denotes the percentage of seeds (by weight) belonging to the variety under certification].

Equipment / Materials required:

1.	Magnifying Glass	**6.**	Analytical Balances	**11.**	Test Tubes
2.	Purity Work Board	**7.**	Weighing Table	**12.**	Funnels
3.	Cupboard	**8.**	Working Table	**13.**	Spatula
4.	Shallow Trays	**9.**	Forceps	**14.**	Sieves
5.	Spoons	**10.**	Blower		

Physical Purity Analysis Components:

The working sample is closely examined with the help of physical Purity Board to

classify it into the following components:

1. Pure seed: Seeds of the variety under certification.

2. Seed of other varieties of the same crop: Seeds of different varieties of the same crop other than the seeds of variety under certification.

3. Seeds of other crops: Seeds of plants being grown as crops other than the crop under certification.

4. Seeds of weeds/objectionable weeds: Seeds, bulblets or tuber etc of plants recognized as weeds under laws, official regulation or by general usage is considered as either seeds of weed or objectionable weeds.

5. Inert Matter: Sand, straw, stones, debris, soil particles, seed without seed coat, empty glumes, lemmas, sterile florets of grasses and cereals etc, are considered as inert matter.

6. Defective seeds: A broken and shrunken seed which is smaller than half of the original size is classified as defective seed (inert matter). A broken seed which is larger than half of the original size and has intact embryo is classified as a pure seed.

Procedure for the physical purity test on weight basis:

The physical purity analysis shall be made on a working sample taken from the submitted sample.

(1) The working samples are spread on the working table and each component is judged individually for shape, size colour, surface texture and / or appearance in transmitted light.

(2) All the impurities as mentioned earlier are removed leaving only the pure seed.

(3) After separating all the components, they should be weighted in grams to the minimum number of decimal points as given below.

Weight of the working sample (g)	Weight of components should be noted in the following decimal point	Example (g)
Less than 1	4	0.3036
1 to 9.999	3	3.036
10 to 9.999	2	30.36
100 to 9.999	1	303.6
1000 to 9.999	0	30456

(4) Calculation of purity percentage:

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

* The physical purity test is done on two or more samples from the same lot as replicate samples to get accurate and reliable results.

Worked Example

The following observations were recorded from the two analytic samples A and B taken from wheat bags and analyzed for purity.

Sr. No.	Components	Weight (g)	
		Sample A	**Sample B**
1.	Weight of the sample	150	150
2.	Weight of inter matter	4.5	5.2
3.	Weight of other seeds	3.2	1.8
4.	Weight of the defective seeds	1.8	2.7
Total amount of impurity = (2)+(3)+(4)		= 4.5+3.2+1.8 = 9.5 g	= 5.2+1.8+2.7 = 9.7 g
Weight of pure seeds		= 150-9.5 = 140.5 g	= 150-9.7 = 140.3 g
Purity % = Weight of pure seed x 100 / Total weight of working sample		= (140.5/150)x100 = 94 %	= (140.3/150)x100 = 93.5 %
Average purity percentage of both samples		= (94.0 + 93.5)/2 = **93.75**	

Note: If the difference in purity percentage of the samples (form same sources) is high (more than 1 %) the fresh samples are to be taken and the test is to be repeated

PRACTICAL NO.7

OBJECT;GERMINATION ANALYSIS AND SEEDLING EVALUATION OF CROP PLANTS

What is Seed Germination?

The emergence and development from the seed embryo of those essential structures which for the kind of seed tested indicate the ability to develop a normal plant under favorable conditions in soil (ISTA, 1985). OR The process by which the dormant embryo wakes up and begins to grow is known as seed germination.

General Principles:

1. Germination test is the most important quality test in evaluating the planting value of the seed lot.
2. Germination test is conducted with seeds from the pure seed fractional of a purity test.
3. At least four hundred seeds are required to conduct germination test.
4. No pre treatment is to be given to the seed except those recommended for breaking the hard seed coat or dormancy.

Types of Seed Germination:

***Epigeal Germination:**

• In this type of germination, the cotyledons are raised above the ground where they continue to provide nutritive support to the growing points.
• Epigeal germination is characteristics of bean and pine seeds.
• Epigeal germination considered evolutionary more primitives than hypogeal germination.

***Hypogeal germination**

• In this type of germination, the cotyledons or comparable storage organs remain under the soil, while the plumule pushes upward and emerges above the ground.
• In hypogeal germination, the epicotyl is the rapidly elongating structure.
• Hypogeal germination is characteristics of pea and gram seeds, all grasses and many other species.

General requirements for seed germination:

a) **Substrata:**

The substrata serve as the moisture reservoir and surface or medium on which the seeds can germinate and the seedling grow. The commonly used substrata are paper, sand and soil.

b) **Adequate water or moisture**:

Moisture is supplied to seeds through the substratum. Water should be free from organic or inorganic impurities. Its pH should be 6.0 to 7.5.

c) Favourable Temperature:

Germination of seeds occurs under different ranges of temperature provided the seed is given adequate moisture. For seeds of various crop species, temperature requirement for germination is different and given in **Table 7.1**.

d) **Light:** Some seeds such as lettuce, tobacco etc. requires light during germination test.

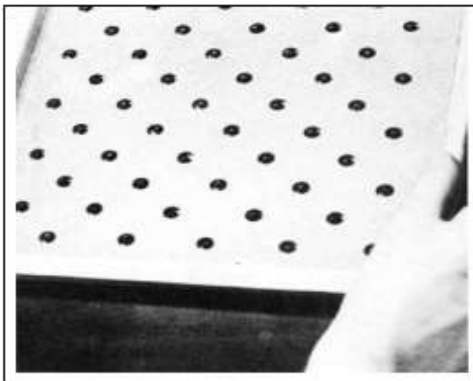
e) **Seeds:** Seeds of a crop variety under germination test.

Table 7.1: Germination methods, temperature and days for first and final count requirement for important crop seeds.

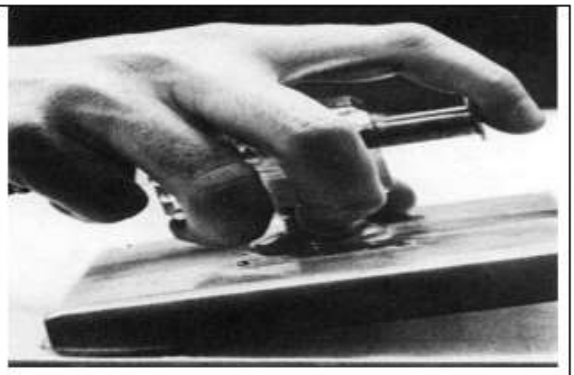
Crops	Substrata	Temperature (°C)	1st Count days	Final Count days
Wheat	TP, BP, sand	20	4	8
Okra	TP, BP	20-30	4	21
Groundnut	BP, sand	20-30,25	5	10
Onion	BP, TP, sand	15-20	6	12
Mustard	TP	20-30	5	7
Pigeon pea	BP, sand	20-30,25	4	10
Chilies	TP, BP	20-30	7	14
Brinjal	BP, sand	20-30,20	5	8
Chickpea	BP, sand	20-30,25	5	7
Green gram	BP, sand	20-30,25	4	12
Cotton	BP, sand	20-30,25	4	7
Maize	TP, BP	20-30	5	14
Tomato	TP	20-30	7	16
Tobacco	TP, BP, sand	20-30,30	5	14
Paddy	TP, BP	20-30	3	7
Pearl millet	BP, sand	20-30	7	4
Castor	TP	20-30	3	6
Sesame, Sorghum	TP, BP	20-30,25	4	10
	TP= Top of paper, BP = Between paper			

Apparatus used for germination test (Fig. 7.1 & 7.2):

1. Seed counting Board or computerized Seed Counter or Vacuum Counter.
2. Seed Germinator or Germination Chamber.

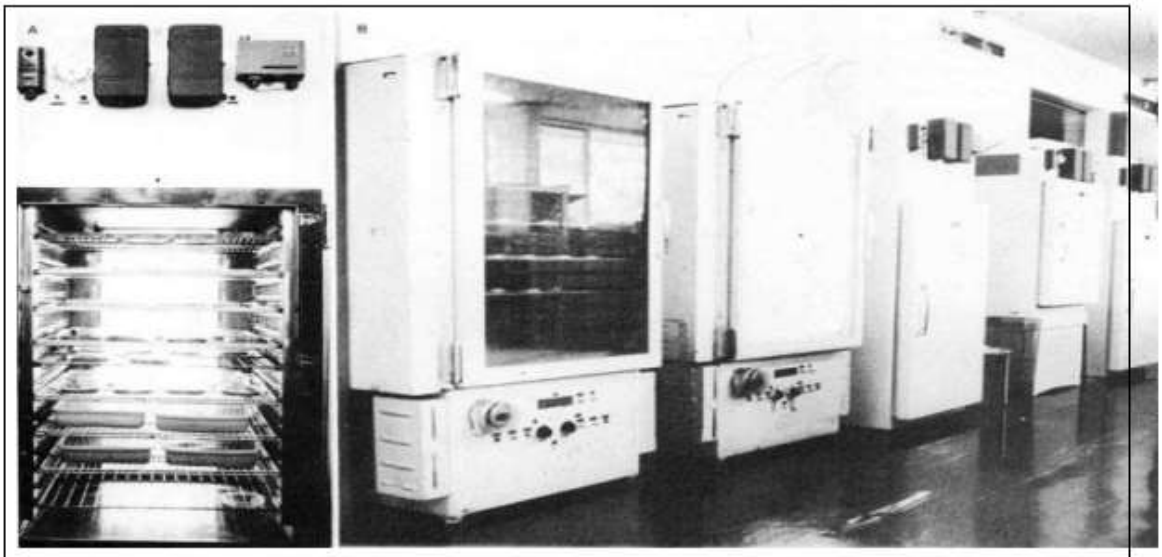


Seed counting Board

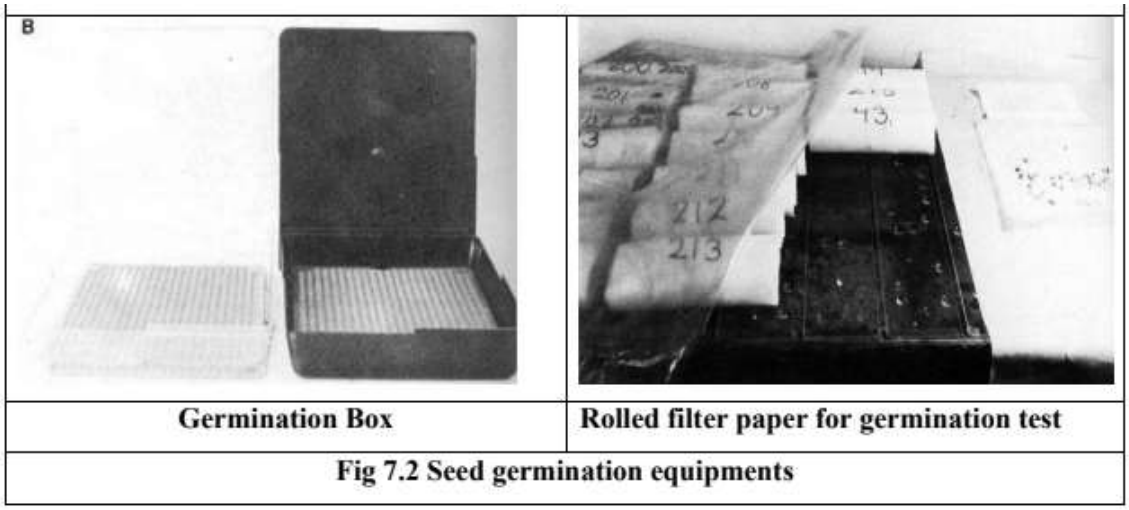


Counting head on a vacuum seed counter

Fig. 7.1 Seed Counter



(A) Open seed germination cabinet **(B) Group of cabinets.**



Germination Box	Rolled filter paper for germination test

Fig 7.2 Seed germination equipments

General Procedure for germination test:

1) The working sample for germination test consists of 400 pure seeds randomly drawn either manually or with the help of counting device such as counting board or seed counter should be tested in replicates of 100 seeds.

2) The seeds counted and evenly spaced on the germination substrate.

3) Temperature must be maintained within 1°C of prescribed limit when using alternating temperature for germination e.g. in groundnut, the test is conducted at low temperature for 16 hrs and at high temperature for 8 hrs per day.

Methods for germination test:

There are mainly three methods for seed germination test based on substratum used.

1. Germination in paper substratum:

(a) Top of paper (TP) (b) Between paper (BP)

2. Germination in sand substratum:

(a) Top of sand (TS) (b) In sand (S)

3. Germination in soil substratum:

1. Germination in paper substratum:

Criteria for selection of paper:

a) Several types of absorbent paper are available for germination test. It should be of good quality and free from toxic substances. The size of paper towel may be 46 cm x 29 cm.

b) Select paper that is relatively cheap, easily available and strong enough to withstand handling and the weight of the seed when damp.

c) The paper must be free from chemical residues that could interfere with the germination of the seeds.

d) The most common papers used are toweling, filter paper or rice straw paper.

e) Filter paper and paper toweling are more expensive. Rice straw paper is cheap but fungal spores from the plant material may cause contamination unless heat-treated before use.

f) The towel may be soaked in water for 2 to 6 hours to moisten it evenly and to remove water soluble toxic substance if present.

g) Before putting seeds for germination on the paper, it must be ensured that the paper is not very wet.

❖ There are two ways to test germination of seeds by using paper substratum: (a) **Top of Paper (TP)** method and (b) **Between paper (BP)** methods.

a) **Top of Paper (TP) method:**

Procedure:

1. Cut the paper to the size and shape of the Petri dish or germination box or a tray fitted with lid.
2. Place a layer of paper in each Petri dish. If the paper is too thin use a double layer.
3. Label the top and bottom of each Petri dish with the accession number, number of the replicate and date of the test.
4. Moisten the papers with good quality water.
5. The thickness of paper used should be more than 2 mm when moist.
6. The water used must be reasonably free from acid, alkali, organic material or other impurities. It can be either tap, distilled or de-ionized water.
7. Seeds are placed directly on one or more layers of moist filter papers or blotters either in a petri dish, germination box or a tray fitted with lid to restrict moisture loss e.g. small seeded species.
8. Arrange the seeds in a regular equidistant pattern on the surface of the paper and placed inside the germination cabinet.
9. Add more water if required.

❖ **Advantage:** seeds can be observed through the transparent lid and the germinating seeds are easy to count.

b) Between Paper (BP) method:

Procedure:

1. Cut the paper to a convenient size to hold one replicate of the seeds when spaced at regular intervals. If the paper is too thin, use a double layer.
2. Label each sheet on the outside of the paper at one end with the accession number, replicate of the test and the date of the test.
3. Moisten the paper with water.
4. Arrange the seeds at regular intervals on the paper, leaving at least two centimeters clear from the edges all rounds.
5. Leave enough space around the edges of the paper so that the paper can be folded back without damaging the seeds.
6. Cover the seeds with another sheet of paper and fold in the edges to prevent the seeds from falling out.

7. Normally 100 seeds are placed on two wet towels and one towel is placed over the seeds. Thus, the seeds are sandwiched between the paper towels.

8. Folding the edges is especially important for large spherical seeds which tend to fall out because of their weight and shape.

9. Roll the paper loosely towards the end with the label.

10. Do not roll the paper too tightly because the compression and lack of oxygen cause the seedlings to develop contorted roots and shoots.

12. Oxygen is essential for respiration during germination. Therefore, any containers used should be adequately ventilated.

13. Keep the paper moist with water if necessary.

❖ **Advantage:**

i) The between paper method is cheap and easy to prepare the samples for germination test.

ii) The use of new paper at each test has the advantage that fungal contamination cannot be carried from one test to another test.

❖ **Disadvantage:** The seeds cannot be observed without unrolling the paper.

II) Germination in sand substratum:

Germination in sand is especially useful for large seeds which are too large to be germinated in petri dishes or too heavy for the between paper method. Sand should be washed, free from toxic materials and sterilized. The sand particle size should range between 0.05 to 0.8 mm in diameter (Fig. 7.3). The amount of water to be added to the sand is calculated as follow:

$$\text{Amount of water (ml) to be Added to every 100 g of sand} = \frac{\text{118.3 ml sand}}{\text{Weight of 118.3 ml sand (g)}} \times 12.2$$

Generally, there are two methods using sand substratum:

i) Top of Sand (TS): The seeds are pressed into the surface of the sand.

ii) In sand (S): The seeds are placed on a leveled layer of moist sand.

Procedure:

1. Pack clean sterile sand into post or trays with drainage holes at the bottom.

2. Water the sand until it is moist. Do not use excess water.

3. Fine sand should be used. Make sure that the sand is clean by sterilizing

before use.Quarry or river sand is better than shore sand, which must be washed thoroughly to remove all the salts.

4. Make holes in a regular equidistant pattern at about the same depth to maintain uniformity of test for each replicate. Ideally, the distance between holes should be at least three to five times the seed diameter.

5. Prepare a label with the accession number, date of sowing and replicate of the test and place in each pot or tray.

6. Fill seeds from each replicate into the holes and cover with sand.

7. Water the sand again to cover the seeds, but do not make it too wet.

8. Sprinkle with water slowly, so that the seeds do not float out from the holes and become mixed. Bottom watering is better than top watering.

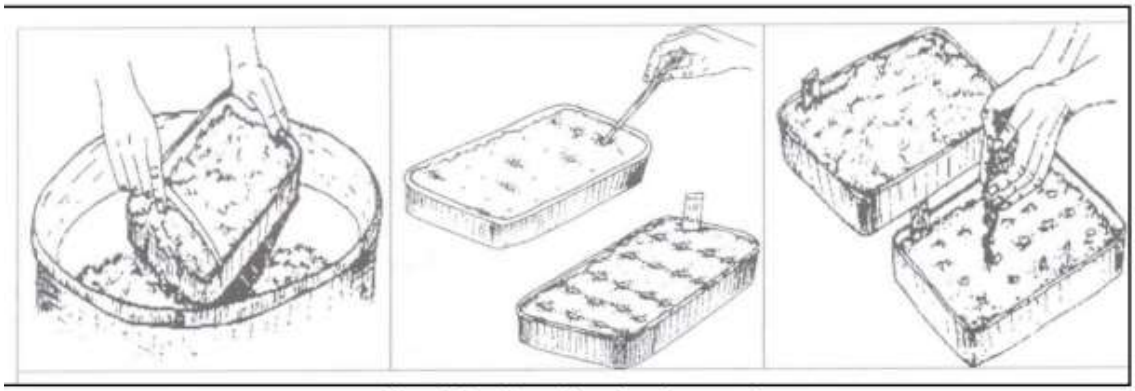


Fig. 7.3: Germination in sand

Types of seedling:

The seedlings for germination test are classified into the following categories:

(1) Normal seedling:

Seedlings which show the potentiality for continued development into normal plants when grown in good quality soil and under favorable conditions of moisture,temperature and light are called normal seedlings. In addition, the normal seedlings must be conformed to one of the following categories.

(a) Intact seedlings:

Seedling with all the essential structures (like root system, shoot axis,

elongated hypocotyls and epicotyls, etc) well developed and complete in all respect showing the proportionate growth and healthy are classified as intact seedling.

(b) Seedlings with slight defects:

Seedling which show certain slight defects in their essential structures (like primer root with limited damage or slight growth retardation, hypocotyl, epicotyl, mesocotyl, cotyledons, primary leaves/leaf, coleoptile with limited damage) provide they show an otherwise satisfactory and balanced development comparable to that of intact seedlings are classified as seedlings with slight defects.

Abnormal seedling:

The seedlings which do not show the potentiality to develop into a normal plant, when grown in good quality soil and under favorable conditions of moisture, temperature and lightare called abnormal seedlings because one or more of the essential structures (like primary roots, hypocotyls, epicotyl, mesocotyl, cotyledons, primary leaves/leaf, coleoptile, seedling as whole deformed, etc.) are defective.

Recording and reporting of germination test:

• The results of the germination test are recorded on the analysis cards of a suitable format. The seed analysts should interpret the result and decide whether the results to the four replications are within the permissible limit of tolerance before reporting the results.

• The result of germination test should be calculated on the basis of the percentage of normal seedling and should be reported in whole number. The percentage of hard seed should be reported separately. The percent of germination is calculated as under:

Germination (%) = Total number of seeds germinated / Total number of seed planted X 100

Retesting:

If the results of the germination test are considered unsatisfactory, it shall not be reported and a second test shall be made by the same method or by alternate method.

Report From:

Crop:	Variety:	Seed sample No.:		
Date of Planting	Replications:	Temp °C:		
Substrata:	Date of observation:			
Category:	Replication:	Total:	Average:	Remark:
1. Normal seedling:				
(i) Intact:				
(ii) Slight defect:				
2. Abnormal seedling:				
3. Ungerminated seed:				
(i) Hard:				
(ii) Fresh:				
(iii) Dead:				

PRACTICAL NO.8

OBJECT; SEED VIABILITY TEST

What is Seed Viability?

According to the seed technologists and commercial men, “a seed is capable for germination and growth for producing a normal seedling” or “the property of the seed that enable into germination under condition favorable for germination” is termed as viability of that seed, provided that only dormancy in the seed remove before the germination test.

Factors affecting seed viability:

A) Pre-harvest conditions:

1. Quality of initial seed
2. Fertility status of the soil
3. Temperature and photoperiod requirements
4. Moisture status of soil at the time of harvesting of seed

B) Harvesting and processing conditions:

1. Mechanical injury to the seed during harvesting
2. Seed size and shape, thickness of seed coat, structural and relative positions of embryo, etc.
3. Seed moisture content at the time of harvesting and processing of seed

C) Post-harvest / Storage condition:

This is the key consideration in seed technology with respect to relative short term strong between harvest and strong time of the longer term requirements of a genetic resources seed gene bank. The three main parameters of the storage conditions which determine seed survival are:

1. Seed moisture
2. Temperature
3. Atmospheric oxygen concentration

Methods for determining seed viability:

There are various tests for determination of seed viability as listed below:

1.	Tetrazolium test	**5.**	Excised embryo test
2.	Germination test	**6.**	X-Ray test
3.	Biochemical test	**7.**	Free fatty acid test
4.	Conductivity test		

1. Tetrazolium (Tz) test:

❖ Tetrazolium test is widely used as a quick and accurate method for determining seed viability and vigour of field and horticultural crops.

❖ This test is accepted as an official test for certain tree seed species.

❖ This test was developed by Professor Georgehakon (1940) in Germany when he was trying to distinguish between live and dead seed by exposing them to Sodium Salt.

❖ In this method, a chemical called 2, 3, 5-triphenyl tetrazolium chloride is used to judge the viability of seeds.

❖ The viable seeds take red stain while unviable seeds are colourless.

Mode of Action of Tetrazolium Test:

In this test, reduction process takes place in living cells and made visible by the reduction of an indicator. The indicator used in this test is a colourless solution of the Tetrazolium salt.

Tetrazolium chloride or bromide which is imbibed by seed within the seed tissues, it interferes with the reduction process of living cells and accept hydrogen from dehydrogenase. By dehydrogenation of 2, 3, 5-triphenyl tetrazolium chloride or bromide, a red stable and non-diffusible compound "triphenyl formazan" produces in living cells. This makes its possible to distinguish the red coloured living parts of embryo from the colourless dead ones.

2, 3, 5-triphenyl tetrazolium Triphenyl Chloride or bromide (colourless)	=	2H / Dehydrogenase group of enzymes	=	Triphenyl Formazan (Red)

Why is tetrazolium test designated as the topographical tetrazolium test?

Since the reaction takes place within the respiring (living) cells and produced formazan which is red, stable and non-diffusible and a clear topography of living and non-living areas within the seed can be developed by using this procedure. For this reason, test is designation at the topographical tetrazolium test.

Apparatus:

1. Conditioning media: germination blotting/filter paper and paper towel.
2. Dissecting devise: Needles, forceps, knives, blades, etc.
3. Staining dishes: Watch glass, petridishes, beakers, etc.
4. Medical dropper: For removing tetrazolium solution after test is completed.
5. Magnifying devise: A suitable hand lens and a stereoscopic microscope.
6. Tetrazolium salt: 2, 3, 5-triphenyl tetrazolium chloride or bromide (TTC or TBB).

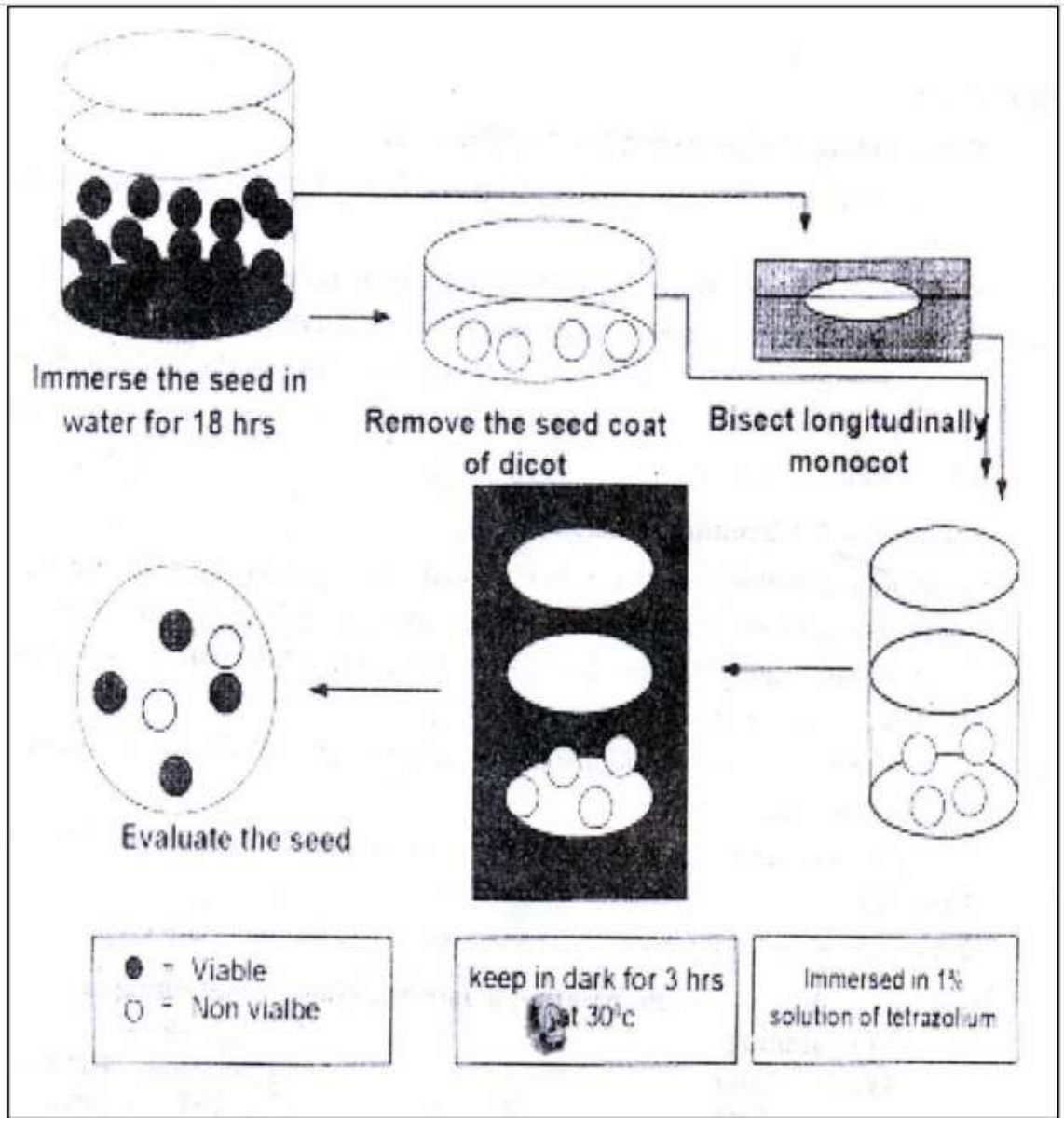


Fig. 8.1: Tetrazolium test for seed viability

Procedure:

(A) Conditioning and preparing the seed (Fig. 8.2):

1. At least 200 randomly pure seeds should be tested in replicate of 100 seeds or less.

2. Seeds should be soaked in water overnight at room temperature.

3. The water soaked monocot seeds are then cut longitudinally (e.g. wheat, maize) or laterally (e.g. small seeded grasses) to express the embryo. Seed coats of dicot should be removed to facilitate the quick penetration of

tetrazolium (e.g. gram).

(B) Staining in tetrazolium solution:

1. After preparing the desired number of seeds, they should be soaked in one per cent tetrazolium solution (pH 6 to 7) at above 30°C for 3-4 hours.

2. Solution with high pH value develops darker stain, while with low pH value develops weaker stain.

3. If the acidity of the tetrazolium solution is higher, the colour will not develop even with a viable embryo.

4. Methods for conducting tetrazolium test for a few important crops are given in Table 8.1.

Table 8.1: Methods for conducting tetrazolium test for important crops.

Crop	Pre-moistening		Preparation before staining	Staining at 30°C	
	Type	Time (hrs)		Solution (%)	Time (hrs)
Rice	BP and Water	18	Bisect longitudinally through embryo and 3/4 endosperm	0.5	3
Wheat	BP and Water	6-18	Bisect longitudinally through embryo and 3/4 endosperm	0.5	2-4
Maize	BP and Water	18	Bisect longitudinally through embryo	0.5	2-6
Gram	Water	18	Remove seed coat	0.5	3-5
Cotton	Water	6-18	Remove seed coat	0.5	2-6

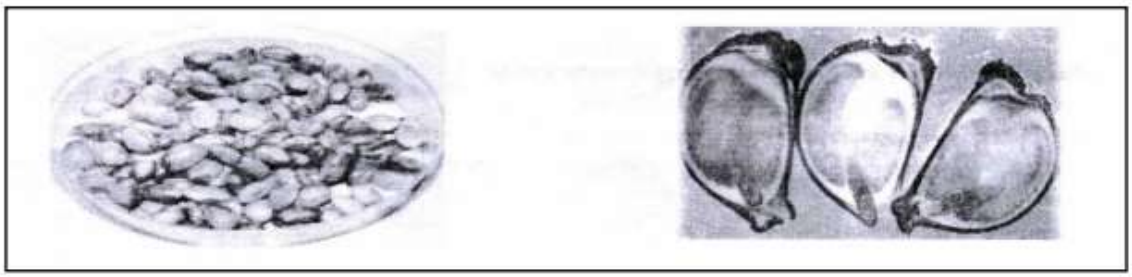


Fig. 8.2: Staining of seed by tetrazolium test

(C) Examination of stained seed (Fig. 8.3):

1. After the complete staining the Tz solution should be drained and de-stained.
2. Seed should be washed thoroughly with water2-3 times and evaluated.
3. Since the tetrazolium solution is photo-reducible, if it is not removed after the staining period, the test is spoiled.

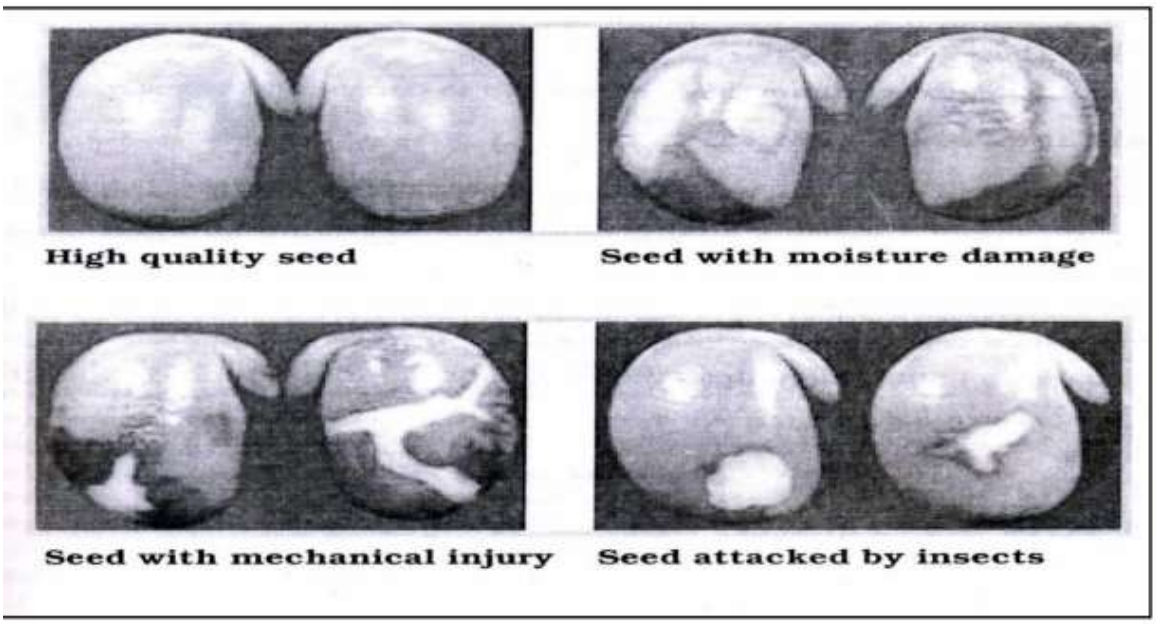


Fig. 8.3: Examination of stained seed by tetrazolium test

(D) Interpretation of staining pattern (Fig. 8.4):

1. Knowledge of seed and seedling structure.
2. Understanding the mechanism of the test and its limitation.
3. Combining interpretation of staining pattern with other visible aspects of seed quality.

4. Interpret the result based on experience.

Advantages of TZ test:

1. Quick estimate of viability can be obtained (within 12-20 hrs.)

2. Seeds are not damaged (in dicot only) in test, therefore, they could be germinated.

3. This test is used even with dormant seeds.

4. Close examination of the individual seeds reveals the reason for proper germination result.

5. No sophisticated equipments are required to carry out the test.

Disadvantages of TZ test:

1. It is difficult to distinguish between normal and abnormal seedlings.

2. This test is not applicable to differentiate between dormant and non-dormant seeds.

3. Since the TZ test dose not involved germination, microorganisms harmful to germinating seedling are not detected.

4. Evaluation of staining pattern requires considerable skill and experience.

Evaluation:

The seeds are evaluated with the help of magnifying devices. Individual seed is evaluated as visible or dead on the basis of staining pattern in embryo followed by cotyledons.

(A)	**Assessment on the basis of staining embryo:**	**Results**
	Embryo completely stained	- Viable
	Embryo unstained	- Non viable
	Plumule or radical unstained	- Non viable
(B)	**Assessment on the basis of cotyledons:**	
	Complete staining	- Viable
	No staining	- Non viable
(C)	**Assessment on the basis of necrosis:**	
	Unstained tissues at the attachment of embryo	- Non viable
	Unstained tissues are away and not connected with embryo	- Viable
(D)	**Assessment on the basis of colour intensity:**	
	Dark red	- Vigorous seed
	Pink colour	- Weak seed
	Dark red fractured	- Non viable

Calculation: Per cent viable seeds in relation to total seeds tested are to be calculated.

PRACTICAL NO.9

OBJECT;VIGOUR TESTS IN CROP PLANTS

What is Seed Vigour?

Seed vigour: Sum total of those properties of seed which determine the activity (i.e. Rapid and uniform production of healthy seedlings) and potential level of performance of seed lots of acceptable germination in a wide range of field conditions is called seed vigour.

Objectives: To determine seed vigour of a given sample:

Why is vigour testing important?

Vigour testing is an important component of seed testing because it's more sensitive test than germination, and becasue loss of vigour may be noted much earlier than loss of germination.

What causes vigour loss?

Vigour loss is due mainly to seed deterioration and aging, which starts as soon as the seed becomes physiologically mature. It's imperative, therefore, that seed be handled carefully to prevent accelerated reduction in performance through physical damage to cell membranes.

Other associated detrimental factors include:

• enzyme activity in immature and dormant seeds;

• respiration during harvest and storage;

• impaired protein and the use of protein and RNA synthesis during periods of low temperature stress;

• genetic damage;

• accumulation of toxic metabolites.

Methods of measuring seed vigor

The general strategy of determining seed vigor is to measure some aspects of seed deterioration or weaknesses, which is inversely proportional to seed vigor.

Below is a brief description for some of the most common seed vigor tests that are used for various crops including corn, soybean, field beans, peas, grasses, vegetable seeds, and other crops.

(A) Physical test:

1. Seed size: One hundred seeds drawn randomly and weighed in gram. The

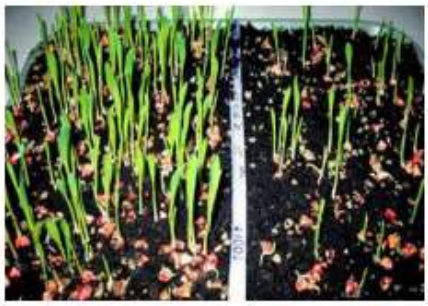
seed lot with high seed weight is considered as vigorous.

2. Seed Density (cm3): Kerosene oil is placed in a graduated measuring cylinder and initial level is measured. Weighed sample is poured in this measuring cylinder and the level of kerosene is again measured. The difference between initial level of kerosene and after placement of seed shows the density of the seed. The seed lot with higher density is considered as vigorous.

3. Physical soundness: Seed lot containing shriveled seeds, undersized, undeveloped, discolored and insect damaged seeds are considered as weak.

(B) Stress Test

1. Cold Test (CT)



The cold test simulates early spring field conditions by germinating the seeds in wet soils (»70% water holding capacity) and incubating them at 5-10°C/41-51°F for a specified period. At the end of the cold period, the test is transferred to a favorable temperature for germination (e.g., 25°C / 77°F in case of sweet corn).The percentage of normal seedlings is considered as an indication of seed vigor. Vigorous seeds germinate better under cold environments.

When can the cold test be used?

1. Select cultivars with the best ability to perform under cold wet soils for early spring planting.
2. Provide basis for adjusting planting rates for individual seed lots.
3. Evaluate the effects of adverse storage conditions, mechanical damage, drying injuries or other causes on seed germination in cold wet soils.

Procedure:

After grinding and properly sieving the soil is filled in tray up to 2 cm depth fifty seeds are placed over the sand and covered with another 2 cm thick layer

of soil. The soil is compacted and enough water is added to make the soil about 70% of its water holding capacity. The temperature of the water should be 100 C. After watering the trays are covered with polythene bags and placed in the refrigerator maintained at 100 C temperature for one week. After one week the trays are removed and placed in the germinator at 250C temperature. The seedlings emerged after 4 days are counted. The germination percentage is computed by counting the number of normal seedlings as in germination lest. Higher the germination percentage greater is the vigour.

2. Accelerated Aging Test (AAT)

The principle of this test is to stress seeds with high temperatures of (40-45°C/130-139°F) and near 100% relative humidity (RH) for varying lengths of time, depending on the kind of seeds, after which a germination test is made. High vigor seeds are expected to tolerate high temperatures and humidity and retain their capability to produce normal seedlings in the germination test.

When can the AAT test be used?

1. Can be used to determine the seed vigor of many crops.
2. Useful in predicting the potential storability of a seed lot.

Procedure:

One hundred seeds each in four replications are tied in a fine muslin cloth. The tied seeds are placed in the jar on a wire mesh. The lower part of the jar is filled with water. There should not be a direct contact between water and seed. The jar is covered with the lead and sealed with paraffin wax to make it airtight. The jar is then placed in the accelerated ageing

chamber maintained at 45 + 20 C temperature for 3-5 days. The jar is removed after this period and the seeds are cooled in desiccators. Seeds are then tested in a normal germination test specific to different crops. The percent germination gives level of seed vigour. Higher the germination percentage greater is the vigour of the seed.

3.Compact soil test:

Seeds are planted in soil at optimum condition in a tray. It is covered with uniform compact layer of the same soil. The seed lot with maximum percentage of emergence under this condition is considered as vigorous.

4.Low or high pH test:

Seeds are planted in soil with low or high p I and kept in optimum temperature in an incubator for germination up to ten day of final count. Lot with high germination percentage is considered as vigorous.

(D) Electric Conductivity Test

This test measures the integrity of cell membranes, which is correlated with seed vigor. It is well established that this test is useful for garden beans and peas. It has been also reported that the conductivity test results are significantly correlated with field emergence for corn, and soybean. As seeds lose vigor, nutrients exude from their membranes, and so low quality seeds leak electrolytes such as amino acids, organic acids while high quality seeds contains their nutrients within well Structured membranes. Therefore, seeds with higher conductivity measurement are indication of low quality seeds as vice versa.

(E) Biochemical test:

1. Tetrazolium (TZ) test:

Principle: The intensity of the red colour in the stained seed during TZ test depends upon the amount of Formosan formed which in turn is regulated by the dehydrogenate activity. In a vigour seed, the intensity of the colour is, higher in more vigorous seed in comparison to less vigorous seed.

Equipment and chemicals: Petri plates, filter paper, incubator, razor, beaker, spectronic 20, distilled water, tetrazolium, methyl allosolve.

➢ The TZ test has been modified in two ways to assess seed vigour:

(a) Aleurone TZ test and

(b) Colorimetrical estimation of Formosan

(a) Aleurones TZ test: Aleurone layer of cereal seed plays an important

physiological role in germination because it produces enzymes which hydrolyse the starch reserves of the endosperm.

Procedure:

1. Seed sample are first washed to remove any traces of fungicides, etc, and then soaked for 16-20 hrs in water at 30°C.
2. The soften seed are cut longitudinally from the distal end towards the base, parallel to the plane of the embryo, leaving two halve joined at the base.
3. Shallow cuts are made through the pericarp of seed half not containing the embryo and the seeds are soaked in 1% solution of Tz salt for 2-4 days at 30°C in covered glass dishes.
4. Some antibiotic compounds (e.g. penicillin and streptomycin) may be added at low concentration to prevent microbial infection.
5. Live Aleurone cells stains red, while dead cells remain unstained.
6. Seeds are classified into group according to the area of stained surface.

No.	Per cent of total Aleurone surface stained	Categories of vigour
i	100 – 75	High vigour
ii	75 – 25	Medium to low vigour
iii	Less than 25	Poor vigour

(E) Seedling Vigor Classification Test (SVCT)

This vigor test is an expansion of the standard germination test (SGT). The normal seedlings obtained from the SGT results are further classified into 'strong' and 'weak' categories. This test has been used for corn, garden beans, soybean, cotton, peanuts and other crops.

The principle of the test

Seedlings have four significant morphological sites for evaluating vigor:

1. Root system.
2. Hypocotyl (the embryonic axis between cotyledons and root).
3. Cotyledons (storage tissue of reserve food for seedling development).
4. Epicotyl (the embryonic axis above the cotyledons).

In this test, seedlings are classified as 'strong' if the above four areas are well developed and free from defects, which is indication of satisfactory

performance over a wide range of field conditions. On the other hand, normal seedlings with some deficiencies such as missing part of the root, one cotyledon missing, hypocotyl with breaks, lesions, necrosis, twisting, or curling are classified as ‘weak’.

Future Role of Seed Vigour Testing

Seed vigour is an important component of seed quality and satisfactory levels are necessary in addition to traditional quality criteria of moisture, purity, germination and seed health to obtain optimum plant stand and high production of crops. The technology of seed vigour testing has not been perfected so far, so much so that there is not a single universally accepted seed vigour test method.

Practical No.10

Object; Grow out Tests and Electrophoresis for Varietal identification.

What is Grow-out Tests (GOT)?

The tests in which appropriate sample of seeds are grown to determine the genetic purity of a given seeds lot of released and notified variety/hybrid in the field and the extent to which the submitted sample conforms to prescribed standards

- Morphological characters are studied in details and compared with authentic sample at proper stage of crop growth.
- The seeds are sown in the main field in a prevailing environment after proper land preparation.
- The check and authenticated samples are also grown along with the test samples.

▪ The crop is grown up to peak flowering and fruiting stage. Based on distinguishable characters, the varieties/hybrids are identified.

➢ **Genetic purity:** It is the trueness of the cultivar, where it should reproduce or resemble its mother plant in all the characteristics.

➢ **Field of applicability:**

▪ Grow-out Test is the official measure for controlling the genetic purity of the seed lot.

It serves as a pre-control as well as a 'post-control' test for avoiding genetic contaminations. According to the official regulations in India, it is pre-requisite for seed certification of hybrids of certain species such as cotton, castor, musk melon and brinjal.

➢ **Sampling:**

▪ The samples for "grow-out test "shall be drawn simultaneously with the samples for other seed quality tests in accordance with the prescribed sampling procedures.

➢ **Size of submitted sample:**

▪ The size of submitted sample for grow-out test for various crops in given in Table-10.1.

Sample Size	Crops
1000g	Maize, cotton, groundnut, soyabean and species of other genera with seeds of similar size.
500g	Sorghum, wheat, paddy and species of other general with seeds of similar size.
250g	Sugar beet and species of other genera with seeds of similar size.
100g	Pearl millet, jute and species of all other genera with seeds of similar size.
250 Propagules	Seed potato, sweet potato and other vegetatively propagating crops i.e. tubers/planting stakes/roots/corms/bulbs etc.

➢ **Size of working sample:**

▪ The number of seeds required for raising the crop to obtain the required number of plants shall depend on the germination percentage of the seed sample and hence seed rate should be adjusted accordingly. (Table 10.2).

Table 10.2: Number of plants required for recording observation in a seed sample of GOT:

Maximum permissible of types (%)	Maximum Genetic Purity (%)	Number of plants required per sample
0.10	99.9	4.000
0.20	99.8	2.000
0.30	99.7	1.350
0.50	99.5	800
1.00 and above	99.0 and above	400

➢**Standard sample:**

▪ The standard sample of cultivar (control) is the official standard against which all other samples of the seed of a particular cultivar will be judged.

▪ The standard sample must not differ significantly in any character and to be obtained

from the originating plant breeder or breeding institute and be stored under controlled temperature and humidity conditions so as to use it each year to sow control plots for cultivar under test.

Method of raising the crop:

▪ Standard and recommended agronomic/cultural practices such as field preparation, size of the plot, row length, distance between the rows, distance between the plants, irrigation, weeding, fertilization, etc., in respect of the specific crop shall be followed uniformly for the sample in question and its control (standard sample).

▪ The germination percentage of the sample(s) in question and the Standard sample must be determined to adjust the seed rate.

▪ Subsequent thinning is not recommended.

▪ Recommended specifications for the above variables are provided in Table10.3 which can suitably modify if considered essential.

▪ The field plots should be grown in two replicates to guard against failure in one part of the field and to reduce environmental and soil fertility variations.

Table 10.3: Recommended specifications for grow-out test:

Sr. No.	Crop	Row length (m)	Spacing (cm)		
			Plant to plant	Row to row	Plot to plot
1	Wheat, Barley	6	02	25	50
2	Pea, Cowpea	6	10	45	90
3	Chickpea, Green, gram, Black gram	6	10	30	60
4	Maize	10	25	60	90
5	Hybrid Cotton	5	10	45	45
6	Paddy				
a)	Very early to medium	6	15	20	45
b)	late and very late	6	25	30	60
7	Pearl millet	6	10	60	90
8	Sorghum	6	10	45	60

Methods for recording observation:

▪ Grow-out test plots must be examined throughout the growing season with emphasis on the period from the flowering to ripening.

▪ The number of total plants and the off-type plants should be recorded.

Calculation and interpretation of the result:

▪ Percentage of other cultivars and species or aberrant found must be calculated up to first decimal place.

▪ While interpreting the results, tolerance should be applied by using the reject number for prescribed standards with reference to sample size as provided in Table 10.4.

Genetic Purity (%)	Reject number for sample size of	
	800	400
99.5 (1 in 200)	08	*
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

* Sample size to small for a valid test.

Reporting of results:

▪ The results of the grow-out test shall be reported as percentage of other species,cultivars or off-type plants.

▪ If the sample is found to be cultivar other stated by the sender, the result shall be reported as such.

▪ Disadvantage of grow-out test:

What is Electrophoresis?

The movement of particles under spatially uniform electric field in a fluid is called electrophoresis.

OR

A technique that separates charged macro molecules (DNA, RNA, Proteins) on the basis of relative migration in an appropriate matrix (such as agarose, polyacrylamide) subjected to an electric field.

I. Agarose Gel Electrophoresis:

▪ Electrophoresis through agarose is the standard method used to separate, identify and purify DNA fragments.

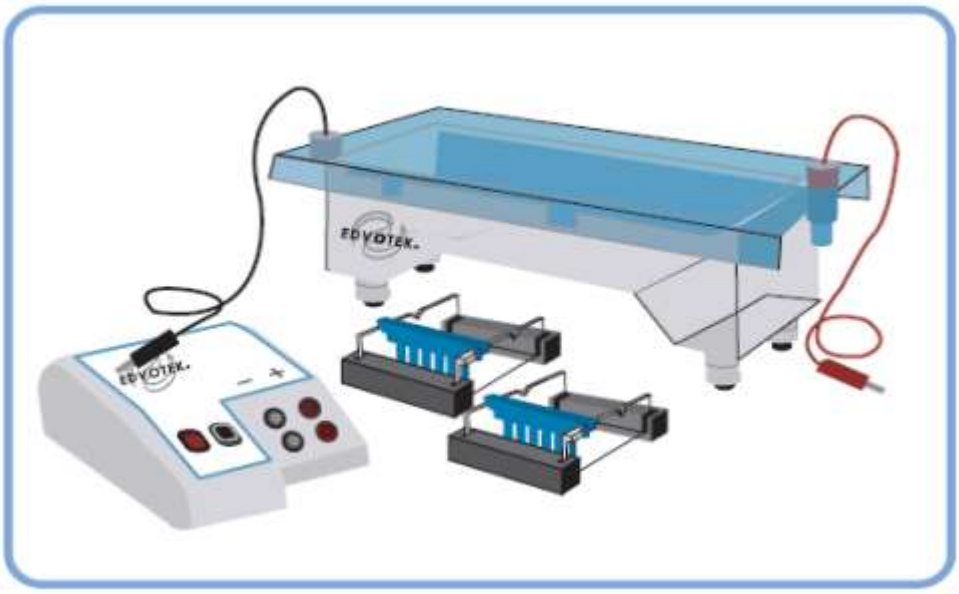
▪ DNA has a negative charge, due to its acidic phosphate groups. Therefore, if an electric current is applied to the DNA, it will move towards the positive electrode.

▪ DNA fragments are separated based on size or molecular weight.

▪ DNA molecules travel through pores in agarose gel.

▪ Each band contains thousands of DNA molecules, all of which are similar in size. The array of bands forms a pattern that is sometimes referred as a

fingerprint.



II. Polyacrylamide Gel Electrophoresis:

▪ It is the latest method of cultivar identification based on protein banding and Isoenzyme activity.

▪ A few seeds are defatted and extracted for protein and isoenzymes.

▪ The extracted proteins or isoenzymes are separated by polyacrylamide gel electrophoresis.

▪ Based on the banding pattern of protein and isoenzymes, the varieties can be differentiated and identified.

PRACTICAL NO.11

OBJECT;GUIDELINES FOR HYBRID SEED PRODUCTION IN CROP PLANTS.

SEED

Any plant part which is used for commercial multiplication of a crop is called seed.The seed of a released and popular variety produced by scientific method is referred to as improved seed or quality seed.

VARIETY

Variety refers to a genotype which has been released for commercial cultivation either by state or central variety released committee. Improved seed plays an important role in maximizing the production and productivity of field crops.

The following steps should commonly be followed while seed production in any crop.

▪ **Selection of seed plot:** Land should be free of volunteer plants, weeds, soil borne diseases and pests. Soil structure should be suitable to the crop grown

for seed production. Soil should be fairly deep, fertile and well drained.

▪ **Selection of seed or seed source:** Source of seed is the main factor for success ofseed production. The seed should be selected of an appropriate class, e.g. Foundation seed for certified seed production.

▪ **Isolation distance**: The minimum separation distance between two crops or crop species or between two varieties of same crop to avoid a chance of cross pollination is known as isolation distance.

▪ **Planting time, planting ratio and seed rate:** Adequate pollen source and synchrony in flowering between male and female lines are very important for successful hybrid seed production. The crop should be planted as per recommended time of planting to avail adequate supply of male flower to pollinate female flower. Depending on the initial moisture status and moisture holding capacity of soil, give one or two irrigation after sowing for seedling establishment for uniform plant stand. Seed rate and ratio of
male: female lines should be maintained. It differs from crop to crop (Table 11.1).

▪ **Rouging:** Rouging is vital step of seed production. This depends on genetic purity of sown seed.

▪ **Fertilizers:** Recommended dose of fertilizers should be applied for raising good crop.

▪ **Irrigations**: Assured irrigation facility must be available for seed production plot. As and when required, irrigation should be given to harvest maximum seed yield from the crop grown for seed production.

▪ **Plant protection measures:** As and when required, recommended plant protection measures should be taken to save the crop from the attack of insect, pest and diseases.

▪ **Harvesting, drying, threshing and storage:** Harvesting is very important operation in seed production because more chances of mechanical mixture during this operation.Seed should be stored at proper moisture content under dry and cool condition. (Table 11.2).

Table 11.1: The field standards for different crops

(A) Minimum field standards (for self and open pollinated varieties)							
Crop	Class of seed	Minimum		Maximum permissible level percentage			
		Isolation distance (m)	No. of inspec tion	Off types	Inseparable other crop plants	Objection able weed plants	Plants/head affected by disease
Paddy	FS	3	2	0.05	-	0.01	-
	CS	3	2	0.20	-	0.02	-
Wheat	FS	3	2	0.05	0.01	-	0.10
	CS	3	2	0.20	0.05	-	0.50
Maize (OPV)	FS	400	2	1.0	-	-	-
	CS	200	2	1.0	-	-	-
Sorghum (OPV)	FS	200	3	0.05	-	-	0.05
	CS	100	3	0.10	-	-	0.10
Pearl millet	FS	400	3	0.05	-	-	0.05
	CS	200	3	0.10	-	-	0.10
Pegionpea	FS	200	2	0.10	-	-	-
	CS	100	2	0.20	-	-	-
Castor	FS	300	2	0.10	-	-	-
	CS	150	2	0.20	-	-	-
Groundnut	FS	3	2	0.10	-	-	-
	CS	3	2	0.20	-	-	-
Mustard	FS	50* 100	3	0.10	*For self compatible & self incompatible types, respectively		0.5
	CS	25*, 50	3	0.50	-	-	0.10
Cotton	FS	50	2	0.10	-	-	-
	CS	30	2	0.20	-	-	-
Maize	FS	400, 600*	4	0.20**	*Pollen shedding, **Seed borne disease (for hybrid)		

(B) Minimum field standards for hybrid varieties)

Crop	Class of seed	Minimum		Maximum permissible level percentage				Remarks
		Isolation distance (m)	No. of inspection	Off types	Inseparable other crop plants	Objectionable weed plants	Plants/ head affected by diseases	
Maize	CS	200. 300	4	0.50	-	-	1.0. 2.0	-
Sorghum	CS	200. 400	4	0.10	0.10	0.10	-	-
Pearl millet	FS	1000	4	0.5	0.05	0.05. 0.02	-	DM ergotted heads
	CS	200	4	0.10	0.10	1.0. 0.04**	-	
Cotton	FS	50	3	0.50	-	-	-	-
	CS	30	4	0.10	-	-	-	-
Castor	FS	300	4	0.50	Male 1.0	Female 1.0	Monocious plant	-
	CS	150	4	1.0	2.0	2.0	2.0	-

Table 11.2: The seed standards for different crops (Minimum seed standards for field crops)

Crop	Class of seed	Minimum					Maximum permissible limit						Remark
		Germination	Pure seed	Inert matter	Other crop seed	Weed seed	Objectionable weeds No/kg	Disease seeds (% by number)	ODV seeds No/kg	Moisture		Other as specified	
										Ordinary pack	Vapour proof pack		
Paddy	FS	80	98	2	10	10	2	0.10	10	13	8	2*	maximum husk less seeds %
	CS	80	98	2	20	20	5	0.05	20	13	8	2	OW: wild rice
Wheat	FS	85	98	2	10	10	2	0.05*	-	12	8	-	SBD: karnel bunt
	CS	85	98	2	20	20	5	0.25*	-	12	8	-	
Maize	FS	90	98	2	5	None	-	-	10	12	8	1.0*	Max. off type ears with off coloured kernels % after harvest.
OPV&Composite	CS	90	98	2	5	None	-	-	20	12	8	1.0*	
Inbred	FS	80	98	2	5	None	-	-	5	12	8	0.2*	
Hybrid	CS	90	98	2	10	None	-	-	10	12	8	0.5*	

Sorgum	FS	75	98	2	10	10	-	0.02*	-	12	8	-	Ergotted seeds scerotia
	CS	75	98	2	10	10	-	0.04*	20	12	8		
Bajra	FS	75	98	2	10	10	-	0.02*	-	12	8	-	Ergotted seeds scerotia
	CS	75	98	2	10	10	-	0.04*	-	12	8	-	
Pigionpea	CS	75	98	2	10	10			20	9	8		
Castor (variety)	FS	70	98	2	None	None	-	-	5	8	5	-	
	CS	70	98	2	"	"	-	-	10	8	5	-	
Hybrid	FS	70	98	2	"	"	-	-	5	8	5	95*	Genetic purity in grow out test
	CS	70	98	2	"	"	-	-	10	8	5	85*	
Ground nut	FS	70	96	4	"	"	-	-	-	9*	5*		For hard shelled kernels
	CS	70	96	4	"	"	-	-	-	9*	5*		For hard shelled kenels
Mustard	FS	85	97	3	10	10	5*	-	10	8	5		Argenomr mexicana.
	CS	85	97	3	20	20	20	10*	-	20	8	5	
Cotton (variety)	FS	65	98	2	5	5	-	-	-	10	6	-	
	CS	65	98	2	10	10	-	-	-	10	6	-	
Cotton Hybrid	FS	65	98	2	5	5	-	-	-	10	6	-	Minimum genetic purity in grow out test 90 %
	CS	65	98	2	10	10	-	-	-	10	6	-	

PRACTICAL NO.12

OBJECT; PROCEDURE FOR SEED CERTIFICATION

As per the provision of seeds act, 1966 and seed rules, 1968, the certification of seeds is done in the following manner :

1. Application for seed certification : All those interested in certified seed production are required to submit an application in prescribed performa to the concerned state seed certification agency along with an application fee Rs. 25/-. The seed certification agency upon receipt of the application will verify the following conditions :

I. That the variety/varieties are notified and eligible for seed certification.

II. That the source of seed is authentic and in accordance with the conditions laid down in the minimum seed certification standards.

III. That there would be no difficulties in reaching the field for carrying out timely field inspections.

IV. That the seed producer is able to provide requisite isolation and the seed field meets the land requirement as per minimum seed certification standards.

V. That the seed processing facilities are available to the seed producer.

2. Seed certification fees : The application on the basis of above verification is accepted by the agency then the applicant has to pay certification fees as under :

Inspection fees includes field inspection, supervision during seed processing, seed treatment, packing, seed sampling, sealing and issue of certificate.

a)	Self pollinated crop	Rs. 325/ ha.
	Hybrid, vegetable crops	Rs. 175/ha.
b)	Seed testing :	Rs. 600/sample
c)	Re processing :	Rs.250/quintal
d)	Re testing :	Rs. 600/sample
e)	Re validation fee :	Rs.20/quintal

3.Inspection of seed fields : Staff of seed certification agency make field inspection at appropriate stages of growth to ensure that the minimum standards for isolation,preceding (previous) crop requirement, roguing are

maintained at all the times and will maintain the records of inspections.

4. Rejecting the field : After completion of inspection season, the staff submits the report of field inspection and problems to the Director of Seed Certification Agency.The board of Directors review the cases and if found not in accordance with the minimum certification standards then officially reject the seed field.

5. Inspection of seed processing : The representative of seed certification agency makes the visit of processing unit as may be required to check the mechanical admixture, seed is cleaned and graded in satisfactory manner, seed is suitably dried, seed treatment is given and seed lot is made homogenous (uniform).

6. Seed sampling : The staff of the agency take samples of all the seed lots which are required to carry the tags. These seed samples are then sent to seed testing laboratory for evaluation of genetic purity, germination and moisture content. If seed lots fail to meet the requisite standard then re-sampling and re-testing is done.

7. Tagging and sealing : After receiving the satisfactory report from official seed testing laboratory, tagging and sealing of seed lots is done under the supervision of the agency staff.

8. Control plot testing : The seed certification agency arrange for a post season grow out test (GOT) and concern the plant breeder to check the genetic purity.

9. Extension of validity period : The extension of validity period of certified seeds shall be for a period of six months and at each subsequent validation as long as the seed confirms the prescribed standards.

10. Revocation of certification: If the certification agency is satisfied that the certificate granted by the agency has been obtained by mis-representation by the seed producer, the agency will give grower a chance to submit causes and if grower does not satisfy the situation then agency will revoke (withdraw) the certificate.

11. Appeal against certification agency : Any seed producer aggrieved by a

decision of a certification agency may appeal against the certification agency to the appellate authority specified by the state government within 30 days from receiving the rejection letter from agency. The appellate authority will discuss the matter critically and pass the necessary order. The decision of the authority is final.