VARIETAL IDENTIFICATION

1. Grow - Out Test

Objective

To determine the genetic purity status of a given seed lot of the notified cultivar / hybrid and the extent to which the sample in question conforms to the prescribed standards.

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**.

The test is required to be conducted for checking the sellers label with respect to genetic purity status of the seed lot **under the provisions of the seeds Act 1966**. In addition grow-out test can also be used as a measure to judge the efficacy of the certification agency or the inspector.

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 samples shall vary according to the species as exemplified in this Table.

Recommended size of submitted sample for Grow-out Test

1,000 g	-	for maize, cotton, groundnut, soyabean and species of other genera with seeds of similar size;
500 g	-	For sorghum, wheat, paddy and species of other genera with seeds of similar size;
250 g	-	Beta and species of other genera with seeds of similar size;
100 g	-	For bajra, jute and species of all other genera;
250 tubers / planting stakes / roots/ corms	-	Seed potato, sweet potato and other vegetatively propagating crops.

Size of working sample

The working sample for grow out test shall be obtained through subsequent mixing and dividing of the submitted sample in accordance with the prescribed procedure for seed sampling.

The minimum population required for taking the observations shall be 400 plants; however, it will also depend on the maximum permissible off-type plants prescribed for the species under consideration in the Indian Minimum seed Certification standards

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.

Number of plants required per sample for grow out test

Maximum permissible off types (%)	Minimum 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 below	400

Procedures

To achieve the accuracy and reproducibility of the grow out test results, the procedures provided hereunder must be followed:

Location of the grow out test

The grow out test shall be conducted in specified areas recommended for the cultivar / hybrid or in off-season nurseries.

Standard sample

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

The standard sample must not differ significantly in any character and be obtained from the originating plant breeder / breeding institute and be stored under controlled temperature and humidity conditions so as to use it each year to sow control plots for cultivars under test. Further quantities of sample must be obtained from the originating plant breeder as and when required. A comparison must be made between the two lots of the standard sample before changing from one standard sample to other.

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 and fertilization, etc., in respect of the specific crop shall be followed both 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. The sowing should be done by dibbling or small plot drill. Seed drill must be carefully checked to ensure its cleanliness. Subsequent thinnings is not recommended. The samples of the same cultivars must be sown in succession and the standard samples are sown at suitable intervals. (one standard sample for every ten sample to be tested).

The size of the plot, row length and spacing shall differ according to the crop. Recommended specification for the above variables are provided in Table mentioned below which can suitably be modified if considered essential.

Recommended row length, distances, spacing for some important crops

S. No.	Crop	Row length (m)	Plant to plant distance (cm)	Space between rows (cm)	Space between plots (cm)
1.	Wheat, barley oats	6	2	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 b) Late and very late	 6 6	 15 25	 20 30	 45 60
7.	Pearl millet	6	10	60	90
8.	Sorghum	6	10	45	60

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.

Methods for taking observations

Grow-out test plots must be examined throughout the growing season with emphasis on the period from the flowering to ripening. All plants must be examined keeping in view the distinguishing characters described for the cultivars both in the test crop as well as the control. While taking the observation, the plants showing deviations in characters against the control should be tagged and examined carefully at a later stage to confirm whether they are off-types or not. The number of the total plants and the off-type plants found should be recorded.

Calculation and interpretation of the results

Percentage of other cultivars, species or aberrants found must be calculated upto first decimal place. While interpreting the results, tolerances should be applied by using the reject number for prescribed standards with reference to sample size as provided in Table.

Reject number for prescribed standards and sample size

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

** indicates that the sample size is too 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 a cultivar other than stated by the sender, the results shall be reported as such.
- If plants of other cultivars are more than 15 per cent, the report shall state that the sample consists of mixture of different cultivars.
- If nothing worthy of special comments is found, the report shall state that the results of the grow-out test of the sample in question revealed nothing to indicate that the name of the cultivar or species stated by the sender is incorrect.

2. Electrophoresis

It is the latest method of cultivar identification based on protein banding and isoenzyme activity. Here single seeds are defatted and extracted for protein and esterases. The extracted proteins or esterases are separated by polyacrylamide gel electrophoresis. Based on the banding pattern of protein and esterase's the varieties can be differentiated and identified.

Electrophoresis for proteins and enzymes: Seeds, seedlings or mature leaves etc. of a crop plant have a specific mix of proteins which are not only crop specific but also variety specific (genotype specific). The electrophoresis in a suitable medium separates the mixture of proteins extracted from seeds, seedlings or mature leaves into distinct bands. Each variety (or genotype) thus has a specific "banding pattern" on the basis of which admixtures of other varieties, differing in "banding pattern" could be detected. This is done by comparing the banding pattern of analysed sample with the standard banding pattern of that variety. The electrophoresis is now being increasingly used for determining the genetic purity of seed samples.

Principle: The term 'electrophoresis' refers to the migration of a charged particle under the influence of an electric field. The movement of ions takes place in a suitable medium, such as, polyacrylamide gel, which acts as a molecular sieve and

cut down convection currents and diffusion, so that the separated components remain as sharp zones with maximum resolution. The separation into distinct bands is due to,

1. differences in the size of molecules (molecular weight) of various proteins. Particles with smaller molecular weights migrate faster than those with higher weights, and

2. differences in charge. The molecules with the higher charge migrate faster than those with a lower charge.

Since proteins carry a net charge at any pH other than their isoelectric point, they migrate in an electric field, the rate of which depends on the charge density (that is, the rate of charge to mass of the molecule). Proteins with higher charge density will migrate faster, thus resulting in differential rates of movement of proteins when a mixture of different proteins is subjected to an electric field. By altering the gel pore size (using polymers at different concentrations) and the charge on the protein molecule (by changing the pH of the system) a high degree of resolution can be achieved for separation of protein molecules in a mixture.