**Topic: Varietal Identification through Grow Out Test and Electrophoresis, Molecular and Biochemical test**

**Lecture 1**

**Objective: Determination of a genetic purity of a seed lot.**

Genetic purity of a seed lot is determined on the basis of distinct morphological characters of the variety expressed at seed, seedling and plant level by comparing its submitted sample with authentic sample under identical environment situation.

Procedure

1. Determination of genetic purty on the basis of expression of distinguishing traits at seed level.
2. Grow-out-Test for determination of genetic purity on the basis of plant morphological characters.
3. **Genetic purity test at seed level**

Objective: To estimate the number of genetic impure seeds

 The seeds of other varieties present in the submitted sample are visually observed on the basis of readily apparent differences in the stable and established seed characters of the variety under test. The authentic seed sample is observed for the expression of morphological traits at seed level. The submitted seed sample is uniformly spread over the surface of the physical purity work board and observations on the character are made. Seeds exhibiting differences in distinguishing characters are sorted out .The seed sorted out as genetic impurity are compared with authentic seed for the expression of morphological traot. The confirmed genetic impure seeds are counted and reported in number. Maximum permissible limit for genetic impure seeds in different crops is presented in Table 1**.**

**2. Grow out test**

 **Objective:** To estimate the genetic purity of a seed lot on the basis of expression of morphological characteristics of the plants produced by the seeds under test.

The seed sample of a seed lot is sowing controlled conditions with authentic sample. Genetic purity is determined on the basis of variation in the expression of distinguishing, stable and uniform morphological characteristics at plant level with reference to authentic sample.

**Sampling**

**Submitted saple:** The submitted sample for grow out test is drawn simultaneously with the submitted sample for other tests. The size of submitted sample varies according to the crop species. The size of submitted sample varies according to the crop species (Table 2).

**Table 2 Recommended size of sample submitted for grow out test**

|  |  |
| --- | --- |
| **Crop** | **Size of sample (g)** |
| Genera with seed size similar to pearl millet | 100 |
| Genera with seed size similar to Beta vulgaris | 250 |
| Sorghum, rice, wheat and other genera of similar seed size | 500 |
| Maize, Cotton, groundnut, soybean and other genera of simiar seed size | 1000 |
| Seed potato, sweet potato and other vegetatively propogated crops | 250 tubers/ planting stakes/roots/corms |

**Working sample:** The size of working sample mainly depends on the test weight and germination percentage of the crop to observe the permissible off type plants prescribed as minimum seed certification standards (Table 3) in the optimum population i.e., minimum 400 plants.

**Table 3 Number of plants required for grow out test**

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

**Location of the grow out test:** The grow out test is conducted in the area where crop can express its marker characters without any variation due to the influence of environment even in off-season or under controlled condition in the glass house.

*Authentic sample of the variety under test:* It is the official sample of the variety obtained from the originating institute and grown in identical situation with sample under test for comparision.

**Land requirement:** in field, test is conducted on land which is free from other crops, wed and volunteer plant seeds with adequate fertility and irrigation facility.

**Observation:** Each and every plant is examined throughout the growing season with emphasis on the expression of stable, uniform and distinguishing marker characterisitics and time of their expression. The plants showing deviation in expression of the characters against the control are tagged and examined thoroughly to confirm their genetic purity. The number of ooftype plants and total; population is counted and recorded.

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

|  |  |
| --- | --- |
| Genetic purity (%) | Reject number for sample size (number of plants) |
| 100 | 400 | 800 | 2000 |
| 99.5 | 00.5 | 02 | 04 | 010 |
| 99.0 | 01.0 | 04 | 08 | 020 |
| 95.0 | 05.0 | 20 | 040 | 100 |
| 590.0 | 10.0 | 44 | 088 | 5200- |

**Reporting result:** Result is reported as percentage of genetic purity.

**Conclusion:** The sample with genetic purity less than the MSCCS is not accepted for sowing purpose.

**Pre and Post control test**: It can be performed as pre control test as well as post control test. For testing of genetic purity of Foundation and certified category of all the varieties and hybrid seed the test is performed as Pre control Plot. Whereas, post Control Test is adopted for testing of Breeder seed. In the event of genetic impurity, the producer used the particular seed for raising Foundation category is informed to rectify the particular problem by rouging based on the informed expression of distinguishing traits.

**Lecture 2**

**Varietal identification through biochemical test**

**Objective:** Verification of genetic purity of cultivars based on variation in expression in response to biochemical tests at seed level.Following are the details of some of the biochemical test performed for different crop species.

1. Fluorescent test
* Seeds of oat are exposed to UV light at 360-380 nm wavwlength on a dark background
* Varieties are classified in two groups on the basis of fluorescent effect r i.e., fluorescent or non-fluorescent
1. Phenol colour test

Varieties of cereals particularly wheat, rice, and sorghum are verified through involvement of the enzyme tyrosinase using phenol as a substance (walls, 1965).

* Seed treated with fungicide are rinsed with methanol prior to soaking
* Seeds are placed in a beaker and immersed in distilled water.
* Two sheets of filter paper are arranged in petriplate.
* Seeds are arranged on filter paper with the help of a pair of forceps keeping the hump portion of the seed upward.
* 1% solution of carbolic acid is applied in each petriplate with the help of pipette till 3/4th part of the seed is covered.
* After application of carbolic acid, the petriplate is covered with lid, immediat3ely to avoid evaporation of carbolic acid.
* The petriplate is placed in an incubator for requires period at prescribed temperature (Table 1).

**Table 1 Details for phenol colour test**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Crop** | **Soaking period****(Hrs)** | **Concentration of phenol** | **Duration of Test (hrs)** | **Temperatur****(oC)** |
| Wheat | 16 | 1 | 4 | 30 |
| Rice | 24 | 1 | 24 | 40 |

* Seeds are observed after prescribed period for development of colour and its intensity to distinguish the genetic impure seed
* Intensity of the color developed on the seed is observed as no reaction (-) deep olive (+), light brown (++), brown(+++) and black(++++).
* Seed pure at genetic level express similar intensity of colour in response to the test. Similarly, any seed showing variation in expression indicates genetic impurity.
* The test is approved for DUS testing of wheat varieties. 20 grains are tested for distinctness whereas, 100 grains for homogeneity with at least two example varieties as control.

**The following are the list of some of the biochemical test for genetic purity of different crops.**

|  |  |  |
| --- | --- | --- |
| **Crop** | **Name of the test** | **Principle** |
| Sorghum | KOH bleach test | On the basis of dark pigmentation present on the tegmen of the seed coat of some varieties due to tannic acid. |
| Identification of colourless seeds of wild rice | KOH test | Two drops of 2 % KOH solution is added in each test tube and colour of the KOH solution is observed after 10 minutes. Development of red colour in the solution confirms presence of wild rice seed. |
| Approved for DUS testing of soybean | Peroxidase activity test | Presence or absence of peroxidase enzyme in seeds of crop from family leguminoceae is under genetic control therefore, variety may be verified based on variation in the expression with the reaction of hydrogen peroxide (Butterry and Buzzell, 1968) |
| Differentiation of white and yellow seeded seeds of *Melilotus alba* | Copper sulphate ammonia test | Seed of Melilotus alba is of white colour whereas, M. officinalis is of yellow colour, but the differentiation in a seed lot on the basis of seed coat colour and expression of other morphological traits of the seed is difficult. Seed of both the species have different expression for copper sulphate ammonia test. |
| DUS testing of Wheat | Coleoptile: Anthocyanin colouration | After one week when leaf is within coleoptiles (stage 9-10) the colour of coleoptiles is observed for presence or absence of anthocyanin pigmentation by naked eye. |
| Cereals, soybean and pigeon pea | Seedling colour | Developent of anthocyanin pigment on the stem of the cereals, soybean and pigeonpea is under the control of genes and a variety can be verified based on pigmentation. |
| Verification of rye grass seed | Reaction of seedling root to UV light | Verification of rye grass seed as of annual or perennial can be done by fluorescent test of the seedling roots. Varieties of annual rye grass (Lolium multiforum) exhibit a positive response due to presence of annuloline, while varieties of perennial ye are non fluorescent. |
| DUS testing of rice varieties. | Amylose content in endosperm | Verification of rice varieties based on amylase content in the endosperm of rice seed. |
| DUS testing of rice varieties | Gelatinization temperature (little et la., 1958) | The test is performed based on alkali spreading and clearing test. |
| DUS testing of rapeseed and mustard | Erusic acid content in the seed | The rapeseed oil contains high amount of long chain fatty acid like erusic acid (C22:1).and eicosenic acid (C20:1). Quantity of erusic acid may reach upto 50-60%.The erusic acid content in the seed of rapeseed and mustard is determined by the procedure of Morison and Smith (1964) to classify the varieties. |

**Lecture-3**

**Varietal identification by electrophoretic mobility of protein on polyacrylaamide gel.**

Principle: Varietal development and its identification is one of the most important aspects of seed industry and seed trade. With the increase in the number of varieties in each crop, it has become difficult to identify and characterize these varieties on the basis of morphological characters alone. It has led to the exploration of new stable characters including genetic makeup to be used as markers for varietal identification.Proteins and enzymes are the primary products of genes and hence are most suited for genetic purity determination. Changes in coding base sequence result in corresponding replacements in amino acids and thus in the primary structure of protein and enzymes. They possess ionizable groups and can therefore be made to exist in solution as electrically charged particles either as cations (+) or anions (-). Molecules with similar charge and size will have differential migration in solution with porous support medium in an electric field based upon difference in net electrical charges as molecules with higher charge migrate faster than those with a lower charge. Particle with smaller molecular weight migrates faster than those with higher weight. This separation of molecules based on their size and net electrical charge is known as electrophoresis.

**Interpretation of protein banding pattern :** After staining of the gel, it is placed over a trans illuminator to see the banding pattern.

Relative mobility of each protein (band) is calculated by the following formula. Distance traveled by protein

Relative mobility (Rm) = Distance travelled by protein

 Distance traveled by tracking dye

On the basis of Rm value and thickness of the band a zymogram is drawn on a paper to show the banding pattern. The varieties are verified on the basis of banding pattern.

1. By measuring Rm of bands
2. Total number of bands
3. Presence or absence of specific band
4. Intensity of band
5. Difference in banding pattern in comparison to authentic zymogram of the variety under test