

15. SEED VIGOUR TESTING

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Seed vigour is an important quality parameter which needs to be assessed to supplement germination and viability tests to gain insight into the performance of a seed lot in the field or in storage. Several definitions have been offered to explain seed vigour. Looking into the complexity of the situation, the ISTA congress in 1977 adopted the definition of seed vigour as "the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence". Although differences in physiological attributes of seed lots can be demonstrated in the laboratory, it was recommended that the term should be used to describe the performance of seeds when sown in the field (Perry, 1984a).

As the germination test is conducted in an optimum condition specific to different species, it is not always possible to get an idea of the performance of a seed lot in the field on the basis of germination test in the laboratory. It is mainly because of the reason that field conditions are seldom optimum and the emerging seedling suffers from one or the other kind of stress. In many cases seed lots having similar laboratory germinations may give widely differing field emergence values. Similarly, two seed lots having the same germination percentage in the laboratory may age differently when stored under ambient condition. These two situations indicate the incompleteness of germination test in assessing the performance of a seed lot in the field or storage. This offers scope and possibility to determine vigour of a seed lot so that its field and storage performance can be assessed.

Seed vigour is still a concept rather than a specific property of a seed or seed lot. Several factors like; genetic constitution, environment and nutrition of mother plant, maturity at harvest, seed weight and size, mechanical integrity, deterioration and ageing and pathogens are known to influence seed vigour (Perry, 1984a). Therefore, care has to be exercised in selecting a seed vigour test to do the job. Two criteria have been employed by the ISTA seed vigour committee to evaluate the performance of seed vigour test methods for different crops :

- (i) Reproducibility of vigour method

- (ii) The relationship between vigour test results and seedling emergence in field soil.

There is no universally accepted vigour test for all kinds of seeds. The determination of following vigour tests will be useful in gaining additional information on seed quality.

I. Growth Tests

Principles : Growth tests are based on the principle that vigorous seeds grow at a faster rate than poor vigour seeds even under favourable environments. Vigorous seeds rapidly germinate, metabolize and establish in the field. Therefore, any method used to determine the rapidity of growth of the seedling will give an indication of seed vigour level.

Apparatus and equipment : All the equipments and materials needed to conduct a germination test are required. Additionally, a top loading balance and an air oven are also required.

Procedure

(a) *First count* : The test is done along with the regular germination test. The number of normal seedlings, germinated on the first count day, as specified in the germination test for each species, are counted. The number of normal seedlings gives an idea of the level of seed vigour in the sample. Higher the number of normal seedlings greater is the seed vigour.

(b) *Seedling growth rate and dry weight* : The seedlings are grown either in laboratory, green house or field. In laboratory, in between rolled towel paper method should be followed. Ten seeds are planted in the centre of the moist towel papers in such a way that the micropyles are oriented towards bottom to avoid root twisting. The rolled towel papers are kept in the germinator maintained at a temperature recommended for crop in reference. After a specified period of time (5-10 days) towel papers are removed and five seedlings are selected, their length is measured and mean seedling length is calculated. Seed lots producing the taller seedlings are considered more vigorous than the seed lots producing shorter seedlings. For dry weight determination, the seedlings are removed and dried in an air oven at 100°C temperature for 24 hours. The seedling dry weight provides additional information for assessing seed vigour.

$$G.I. = \frac{n}{d}$$

d = day after planting

Example

No. of seedlings= 0,0,0,40,30,12,7, counted

No. of seedlings = 0,0,0,0,30,42,21 counted

$$\frac{0}{1} + \frac{0}{2} + \frac{0}{3} + \frac{40}{4} + \frac{30}{5} + \frac{12}{6} + \frac{7}{7}$$

$$= 19$$

$$\frac{0}{1} + \frac{0}{2} + \frac{0}{3} + \frac{0}{4} + \frac{30}{5} + \frac{42}{6} + \frac{21}{7}$$

$$= 16$$

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(d) *Seed vigour index (S.V.I.)* : This is calculated by determining the germination percentage and seedling length of the same seed lot. Fifty seeds each in four replications are germinated in towel papers as prescribed for the crop species in germination test. While evaluating the number of normal seedlings at the time of final count, the seedling length of 5 randomly selected seedlings are also measured. Seed vigour index is calculated by multiplying germination (%) and seedling length (mm). The seed lot showing the higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973).

Example

Seed lot	% germination	Seedling length, mm	Vigour index
A	96	85	8160
B	95	76	7220
C	94	71	6674

In this example seed lot A is the most vigorous and seed lot C the least vigorous as they have the highest and the lowest values of seed vigour index, respectively.

2. Conductivity Test

Principle : Weakening of cell membrane in poor vigour seeds causes leakage of water soluble compounds like sugars, amino acids, electrolytes etc. when immersed in water. On the other hand, fresh seeds having intact membrane leach less quantity of these chemicals. The measurement of electrical conductivity (EC) of the leachate by a good and sensitive conductivity meter gives an accurate estimation of membrane permeability. The EC has been positively correlated with the emergence percentage of peas and broad beans (Mathews and Bradnock, 1968). The value of this test appears to be restricted to the large seed species of the Leguminosae (Perry, 1984b).

Apparatus and equipment : Conductivity meter, beaker, 0.1% mercuric chloride, distilled water, seed sample, wash bottle and tissue paper.

Procedure : A seed sample of 2-5 gram is weighed and surface sterilized with 0.1% HgCl₂ for 5-10 minutes. The sample is washed thoroughly in distilled water. The clean seeds are immersed in 100 ml of water at $25 \pm 1^\circ\text{C}$ temperature for 10-12 hours. After this the seeds are removed with a clean forcep. The steep water left is decanted and is termed as leachate.

The conductivity meter is warmed for about 30 minutes before testing. First the conductance of distilled water is measured in a beaker. The electrode is then cleaned with a tissue paper and conductance of the leachate is read. The electrode is thoroughly washed using a wash bottle and wiped with a clean tissue paper before reusing. While recording the conductance, the lower bulb of the electrode should be fully emerged in the leachate. To get the EC of leachate the reading of distilled water is subtracted from the sample reading. The value is then corrected for the temperature and multiplied by the cell constant factor. The reading is expressed as $\mu\text{mhos/cm/g}$ of seed. Lower the value of EC greater is the seed vigour.

3. Hiltner Test (Brick gravel test)

Principle : The test was developed by Hiltner in Germany in 1917. He observed that the seeds of cereal crops affected by Fusarium disease were able to germinate in regular test but were not able to emerge from brick gravels of 2-3 mm size. Compared to this, healthy seeds were able to emerge from the brick gravel (Robersts, 1972). The principle is that the weak seedlings are not able to generate enough force to overcome the pressure of brick gravels, so this method can be used to differentiate vigour levels in cereal seeds. Perry (1984b) found this method reproducible and associated with field emergence in case of wheat.

Apparatus and equipment : Germination box, aluminium tray, sand, sand marker brick gravel of 2-3 mm size, germinator, seed sample.

Procedure : The sand is sieved, moistured and filled in the germination box leaving about 3 cm empty at the top. One hundred seeds are placed in each box in the impressions made by a sand marker. After this 2-2.5 cm of porous brick gravel is spread over the seeds. The box is kept in the germinator at appropriate temperature. After the period required for germination, the box is removed and the seedlings which have emerged through the brick gravel layer are counted. The percentage of emerged seedlings are used to compare seed vigour of different lots. The test should be repeated 3-4 times to get authentic value.

4. Paper Piercing Test

Principle : The principle of paper piercing test is similar to that of brick gravel test. High vigour seed lots are expected to produce strong seedlings which can pierce a particular type of paper while seedlings of poor vigour lots may not be able to pierce the paper. Therefore, the seedlings which emerge by piercing the paper

are more vigorous than those which are not able to emerge through the paper.

Apparatus and equipment : All the material required for conducting germination test in sand boxes or trays plus the special paper which should have the following characteristics :

- (a) Basic weight = 90 g/m^2
- (b) Thickness = 0.4 mm
- (c) Bulk = 4
- (d) Dry bursting strength = 0.3 kg/cm^2
- (e) Breaking length = 1000-5000 mm
- (f) Filtering speed = 500 ml/minute
- (g) Wet bursting strength = 150 mm
- (h) Ash content = 0.1%
- (i) Fibre composition = Chemical wood pulp with high alpha percentage

Procedure : The cereal seeds are placed on 1.5 cm moist sand in a tray or sand box. The seeds are covered with specially selected dry filter paper, which is then covered with 2 cm of moist sand. After this, the sand boxes/trays are kept in a germinator maintained at 20°C temperature for 8 days. After 8 days sand boxes/trays are taken out and seedlings emerging above the paper are counted. A seed lot having maximum number of seedlings coming out of paper is considered to be most vigorous. *The test is highly dependant on the quality of paper and should be used when such papers are available.*

5. Cold Test

Principle : The cold test has been developed in USA to evaluate the seed vigour of maize (corn). In USA when the corn is planted in late spring, the soil is humid and cold. The weak seeds do not germinate and establish. Therefore, to simulate the actual field conditions witnessed at the time of corn planting, cold test

has been developed. The test aims to differentiate between weak and vigorous seed lots by subjecting them to low temperature prior to germination at optimum temperature. The test has been criticized for using field soil which greatly varies from place to place.

Apparatus and equipment : Aluminium tray, field soil, sand marker, germinator, seed sample.

Procedure : After grinding and properly sieving the soil is filled in tray upto 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 10°C. After watering the trays are covered with polythene bags and placed in the refrigerator maintained at 10°C temperature for one week. After one week the trays are removed and placed in the germinator at 25°C temperature. The seedlings emerged after 4 days are counted. The germination percentage is computed by counting the number of normal seedlings as in germination test. Higher the germination percentage greater is the vigour.

6. Accelerated Ageing Test

Principle : The accelerated ageing test has been developed at the Seed Technology Laboratory, Mississippi State University, USA for determining the storage potential of seed lots. The ageing process is accelerated by subjecting the seeds to high temperature and relative humidity in a chamber before standard germination. The seed lots that show high germination in accelerated ageing test are expected to maintain high viability during ambient storage as well. Thus, ageing test gives an indication of the performance of the seed lot during ambient storage. Tests conducted at Pantnagar with Bragg soybean seeds have shown positive relationship between 3 days accelerated ageing test (42- 45°C temperature, 95-100% R.H.) and viability after 6 months of ambient storage (Gupta, 1980). However, Perry (1984b) reported inconsistency in accelerated ageing test results and not well related to field emergence of maize and soybean. The test also suffers from fungal growth on seeds at high temperature and humidity (Agrawal, 1987). This test is recommended for soybean seeds.

Apparatus and equipment : Accelerated ageing chamber, equipment for germination test, seed samples, tight jar, muslin cloth, wire mesh etc.

Procedure : One hundred seeds each in four replications are tied in a fine muslin cloth. The tied seeds are placed in 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 the seed. The jar is covered with the lid and sealed with parafin wax to make it air tight. The jar is then placed in the accelerated aging chamber maintained at $45 \pm 2^{\circ}\text{C}$ temperature for 3-5 days. The jar is removed after this period and the seeds are cooled in a dessicator. The 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.

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. As agricultural and horticultural techniques become progressively more sophisticated, the need for high vigour seeds will increase and testing standards, similar to those reconginzed for germination will be required (Perry, 1984b). 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. Research is needed to further refine the current seed vigour test methods and to develop new methods which are more related to field/storage conditions.