2.7. TESTS FOR SEED VIGOUR

Seed vigour is defined as 'the sum total of those properties of seed which determine the level of activity and performance of the seed/seed lot during germination and seedling emergence' (A_{non} , 1995). The estimates of good vigour, therefore, would assure successful field establishment of vigorous seedlings maintaining adequate crop volume for good yield even with a reduced seed-rate for sowing. Seed vigour is gradually acquired during development and maturation of seed (Hampton & Tekrony, 1995) and is influenced by factors, *e.g.*:

- (i) Physiological and genetic constitution of the crop variety,
- (ii) Environmental and nutritional constraints of the mother plant producing seeds,
- (iii) Seed size,
- (iv) Infection by pathogens,
- (v) Stage of maturity at harvest,
- (vi) Mechanical injury, and
- (vii) Uncontrolled conditions of moisture and temperature during storage.

Vigour tests do not replace germination tests; but supplement it with more information and should be prognosticated under field condition to detect the differences of seed activity and performance which are not discernible in controlled optimal laboratory conditions for the kind of seed involved. Types of seed-vigour tests are as under :

2.7.1. Physical Tests

(i) Seed size. Seeds are passed by shaking through sieve having standard mesh size to determine the seed size difference. The length, breadth or diameter of large seeds, *e.g.* paddy, wheat, maize, soybean can be measured with the help of graph paper or micrometer. Generally, seeds which are larger than the average seed size of a seed lot have high vigour.

(*ii*) 1000 seed weight. Either the whole working sample is counted in an automatic counter or replications of 125 seeds, or 5 replications of 200 seeds, or 4 replications of 250 seeds are vigour.

Average weight (gm) of 1000 seeds of some crops

Paddy	• -
Wheat	20 - 25
Maize	35 – 45
Groundnut	180 - 200
Soybean	430 - 450
Sesamum	95 - 100
Jesunum	2.7 - 3.0

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Chick pea	185 - 275
Lentil	28 - 30
Mung bean	30 - 32
Urd bean	35 - 40
Pigeon pea	100 - 115
Lathyrus (grass pea)	85 - 90

(*iii*) **Physical soundness.** Fully mature, bold seeds without any crack or damage which can be identified by naked eye or under microscope are the physically sound seeds having high vigour.

2,7.2. Performance Tests

(*i*) First count. The number of normal seedlings which can be removed on first count of germination test indicates the seed lot's vigour. The higher the percentage of normal seedlings removed in the first count the better is seed vigour and quality (Table 31).

TABLE 31. First count of seedlings from germination test for seed vigour

Сгор	Days after planting
Millet, sunflower, linseed, sesame, jute, berseem, radish	3
Barley, sorghum, wheat, maize, pigeon pea, field bean, broad bean,	
urd bean, safflower, sunnhemp, cotton, kenaf, mesta, alfalfa, melon,	
cucumber, squash, pumpkin, okra, beet	4
Oats, paddy, chick pea, soybean, lentil, pea, mung bean, cowpea,	
groundnut, rape, mustard, cumin, cabbage, cauliflower, knol khol,	
tomato, lettuce, turnip	5
Onion	6
Castor, tobacco, brinjal, carrot, chilli, spinach	7

(*ii*) Speed of germination. Normal seedlings are removed and counted daily, until all seeds that are capable of producing normal seedling have germinated. An index or speed of germination is computed for each seed lot by dividing the number of normal seedlings removed each day by the day on which they are removed [from a minimum of 4 replications of 100 seeds and by adding the quotients of daily counts] divided by the number of days of germination (Table 31).

TABLE 32. Speed of germination of seed lot

Seed lot	Number of seedlings removed Days after planting	Index or speed of germination
A	$\frac{0}{1} + \frac{0}{2} + \frac{0}{3} + \frac{12}{4} + \frac{10}{5} + \frac{24}{6} + \frac{28}{7} + \frac{24}{8}$	16
В	$\frac{0}{1} + \frac{0}{2} + \frac{9}{3} + \frac{32}{4} + \frac{50}{5} + \frac{6}{6} + \frac{0}{7} + \frac{0}{8}$	22

Thus lot B with a higher index or speed of germination possesses high vigour than lot A, even though the number of seeds germinated is more in lot A.

SEED SCIENCE AND TECHNOLOGY

(*iii*) Seedling growth rate. Seedling lengths are measured after a number of $day_{s after}$ planting (DAP) as specified for different kinds of crops, *viz*

Crop	Days after planting
lute	5
Second barseem radish	6
Barley, millet, maize, urd bean, mung bean, rape, mustard, sunflower, linseed, lettuce, turnip	7
wheat, chick pea, soybean, pea, cowpea, kenal, mosta, mass	8
Oats, sorghum, pigeon pea, lentil, groundnut, sunnhemp, alfalfa,	10
Catton onion	12
Paddy, broad bean, safflower, castor, chilli, cumin, water melon,	
tomato, brinjal, beet, carrot	14
Tobacco	16
Okra, celery, spinach	21

Six replications each of 20 to 25 seedlings are taken for each sample. The average length of seedlings per sample is calculated. Seed lot with better seedling length than the average is considered to have higher vigour.

(*iv*) Seedling dry weight. After measuring the length, seedlings are cut, dried at 110°C for 17 to 18 hours and weighed. Seedlings having higher dry weight are considered to have higher vigour.

2.7.3. Stress Tolerance Test

(1) Accelerated ageing test. Seeds are exposed at 40 to 50°C at 100% Relative Humidity for 2 to 8 days and sown for regular germination test in laboratory. Seed lot showing good germination even after such rigorous ageing test has high vigour and hence the capacity to perform well in field conditions.

(if) Brick-gravel test. This test was developed by Hiltner & Ihssen (1911) in seeds. A 2 to 3 cm thick layer of porous type of brick-gravel is placed over seeds sown for germination. In case of small grains, about 30 to 40 mm thick layer of moist gravel is placed above the seed. Weak, partially diseased, corkscrew type seedlings and other defective seedlings or those with injured coleoptiles tips cannot emerge through this layer. The seedlings that can emerge are considered to possess high vigour and are expressed in percentage for comparison of seed lots.

(*iii*) **Paper piercing test.** This test is used in place of brick-gravel test to provide a standard impeding cover above the seeds. It utilizes the regular germination testing method with a special type of paper disc placed over the seeds in such a way that only vigorous seedlings will be able to penetrate. The quality of paper selected simulates favourable soil conditions for comparability of results. The test is especially suitable for small grains. Seeds of cereals are placed on top of about 1 to 2 cm moist sand, covered with the paper and about 3 cm of moist sand above it and kept at 20°C for 8 days. The specification of the paper according to ISTA is :

126

SEED QUALITY TESTING

Basic weight	:	90 gm/m ²
Thickness	:	0.4 mm
Breaking length	:	500 ml/mm
Dry bursting strength	:	0.3 kg/cm^2
Wet bursting strength	:	150 mm water
Ash content	:	0.1%

(iv) Cold test. This test is useful to estimate performance of seed from emergence ability to survival and sustenance of normal growth and development of seedling in low soil temperature conditions. Seeds are planted in plastic box or flats on paper towels or in soil collected from field, watered and placed at 10°C for 7 days. After the cold treatment, the tests are transferred to a warmer temperature (25 to 30°C). Seedling counts and/or measurements are made 4 days after transfer. This test was developed for maize seed; but is found to be equally useful for evaluation of cotton, sorghum and soybean seeds as the cold and wet conditions are frequently found to prevail in the field after sowing and prior to emergence of the seedling of these crops. The adverse conditions prevent or retard germination, leaving the low vigour seeds in a vulnerable condition for invasion and destruction by soil microorganisms.

(v) Exhaustion test. It involves germination and seedling development in complete darkness and with carefully regulated amount of water. Seeds are placed on a marked line on moist paper towel (30 to 35 cm \times 23 to 25 cm) which is then folded into a roll and placed in a glass container that is covered to prevent moisture loss. The tests are kept at 10°C for 10 days in dark. Seedlings which develop shoot that extend beyond a line spaced 3 to 4 cm above the seed placement line and roots that extend beyond a line 5 cm below the seed placement line are considered to be strong. The double layer of paper towel is moistened initially with 30 ml of water for cereals; 50 ml of water for maize, peas and beans. The test discriminates between the type of seed weakness or aggressiveness that is reflected in elongation of shoot and roots.

(vi) Cool germination test. A constant temperature of 18° C and sufficient humidity are maintained during germination. The count is taken on 6^{th} or 7^{th} day. This test measures the effect of cool remperature on germination of cotton seed and growth rate of the cotton seedlings.

(vii) Hot water test. Soaking of seeds in water at 40°C for 4 hours before continuing germination test under favourbale conditions is suitable for maize. There is a good correlation between this test and cold test in case of maize.

(viii) Compact soil test. Tests with non-sterile soil at favourable temperature have been proposed from time to time; but have never gained much favour. Difference in texture, structure and micro flora in soil creates problems in standardization and reproducibility of results.

(*ix*) Low and high pH. Use of lower or higher pH than optimal for a particular variety are suitable for testing adaptability or resistance (both of which are components of aggressiveness) to adverse pH conditions of the soil in field for crop growth. A vigorus seed lot will have better growth rate of seedling in such adverse conditions.

(x) Sick soil. Tests are conducted on sick soil which is heavily infested with pathogenic micro-organisms. Better germination and growth rate of seedling will indicate higher vigour.

127

SEED SCIENCE AND TECHNOLOGY

(xi) Wet or dry soil. Tests with higher or lower soil moisture content than optimal $f_{or a}$ variety will adversely affect germination and growth rate of seedling. A seed lot with b_{etter} performance under this adverse condition will have higher vigour.

2.7.4. Biochemical Tests

Abdul Baki (1969) listed four categories of biochemical changes associated with reduction in vigour or germinability, as follows :

- (a) A decline in overall metabolic activity and its manifestation in reduced rate of respiration, slower seedling growth and lower germination.
- (b) An increase in total activity of certain enzymes, e.g. phytase, protease, phosphatase,
- (c) A decrease in activity of respiratory enzymes, e.g. catalaze, peroxidase, dehydrogenase, glutamic acid decarboxylase, etc.
- (d) An increase in membrane permeability resulting into greater leakage of sugars, amino acids and organic solutes from the seed.

Biochemical tests can thus be designed to reveal the unsound or faulty functional areas of seeds affecting germinability and vigour; assess the response of an essential enzyme as an index of general viability and monitor the complex system of function vital for early stages of germination in the seed.

(i) GADA test. It measures the level of enzyme Glutamic Acid Decarboxylase Activity (GADA) by the amount of CO_2 given out and is positively correlated to seed vigour (Grabe, 1964). Significant responses are obtained with wheat and maize; but it has not been proved valuable for other types of crop seeds.

(*ii*) **ATP test.** Adenosine Triphosphate (ATP) content of seed can be determined by Luciferase activity. Luciferin produced in boiling glycerin extract, indicated that the ATP level is higher in seed with more vigour (Lin & Cohen, 1968).

(*iii*) **TZ** test. The same seeds used in Tetrazolium test for seed viability can be used for determination of vigour. The intensity of colour developed and the time taken for development of colour are used for estimation of vigour. Obviously, development of deeper colour indicates more vigour. The intensity of colour can be measured in colorimeter after eluting the colour from embryo. The time taken for development of colour is measured from the instant the split seeds are placed in TZ solution.

(*iv*) **RQ test.** During first few hours of imbibitions of water, respiration rate increases which is highly associated with subsequent seedling growth rate in maize, wheat, paddy, lima bean, etc. In maize and paddy differences in initial respiration rates can be used to differentiate high, medium, and low vigour seeds. During the process of respiration, the ratio of volume of CO_2 evolved to the volume of O_2 consumed per unit time is termed 'Respiratory Quotient' (RQ). RQ is more often related to vigour than O_2 uptake alone. The rate of gas exchange is measured in a respirometer called 'Warburg respirometer'.

(v) **MI test.** Membrane Integrity (MI) test is also known as leaching test. With ageing the permeability of seed membrane increases allowing increased diffusion of sugar, free amino acid and organic acid molecules in water outside. Temperature has pronounced effect on leaching. The concentrations of leachates are normally measured either by Electrical Conductivity method or by

128