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FACULTY OF AGRICULTURAL SCIENCES

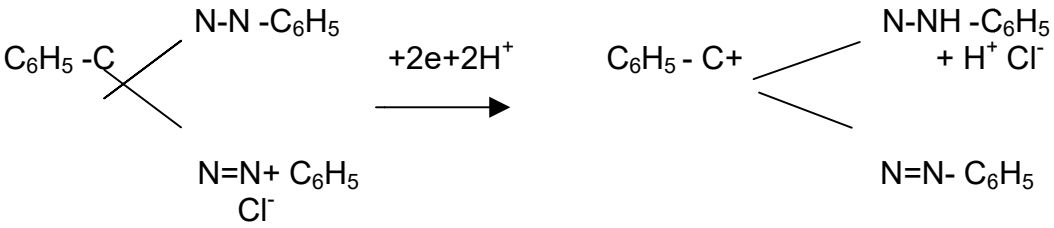
AND ALLIED INDUSTRIES

QUICK VIABILITY TEST

The relative long periods of time required for completion of germination tests delays the seed marketing. This necessitated the development of rapid methods for estimating the germination capacity of seeds. This test was developed by Lakon (1942) in Germany.

Principle

It is a biochemical test, in which living cells are made visible by reduction of an indicator dye. The indicator used is 2,3,5 triphenyl tetrazolium chloride. Within the seed tissues, it interferes with the reduction processes of living cells and accepts hydrogen from the hydrogenases. By hydrogenation of the 2,35 - tri phenyl tetrazolium chloride; a red stable and non difficusable substance, triphenyl formazan is produced in living cells. The reaction is as follows.



2,3,5 triphenyl tetra zolium chloride Triphenyl formazon.

This makes it possible to distinguish red coloured living parts of seeds from the colourless dead ones. Staining of seeds determines whether seeds are to be classified as viable. Completely stained seeds are viable partially and completely unstained seeds are non-viable.

Field of application

This test is not valid for previously germinated seeds.

Method of Tetrazolium testing

A. Testing sample

A representative sample of 50(or) 100 seeds is usually sufficient. However, 200 seeds, in replicates of 100 seeds is recommended.

B. Preparation of solutions

1% solution is used for seeds that are not bisected thro' the embryo, while 0.1% solution is used for seeds in which the embryo is bisected.

The pH of the solution should be between 6 and 8 for best staining. If the pH of the water is not in the natural range, the TZ salt should be dissolved in a phosphate buffer solution. The buffer solution is prepared as follows

Solution -1- Dissolve 9.078 g of $KH_2 PO_4$ in 1000 ml of water

Solution -2- Dissolve 11.876 g of $Na_2HPO_4 . 2H_20$ in 1000 ml water.

Take 400 ml of solution 1 and 600 ml of solution 2 and mix them together. In litre of buffer solution prepared as above, dissolve 10 gms of TZ salt. This gives 1% TZ solution of pH 7.0. This may be further diluted to give lower concentrations.

The solution should be stored in brown bottle to prevent deterioration from light.

Methods of preparation for tetra zolium testing

The seeds are first prepared for staining then stained and evaluated for viability.

Method 1 : Bisect longitudinally

(e.g) maize, sorghum, small grains, large seeded grasses. Soak the seeds in water for 3 to 4 hours. Bisect the seeds by cutting longitudinally thus exposing the mains structures of the embryo. Use one 1/2 of each seed for testing.

Method 2 : Bisect laterally

(e.g.) Small seeded grasses

The seeds are cut laterally near the centre of the seed above the embryo. Place embryo end in TZ solution.

Method 3 : Pierce with needle

(e.g.) Small seeded grasses

Puncture the seeds by piercing thro' the seed into the endosperm near the embryo, but avoid injury to the embryo.

Method 4 : Remove seed coat

(e.g) Dicots with seed coats impermeable to tetrazolium.

Soak the seeds in water for 3-4 hours and then the seed coats and place the seeds in the TZ solution. In some crops like cotton a thin membrane adhering to the cotyledons is also removed in addition to the seed coat.

Method 5 : Conditioning only

(e.g) Large seeded legumes

Seeds of soybeans and other large seeded legumes may swell so rapidly and irregularly when placed directly in water or TZ solution that the seed coats burst. Hence, it is preferable to condition these seeds slowly in moist paper towels overnight before staining, so that they absorb moisture slowly without any damage to the seed.

METHOD 6 : NO CONDITIONING OR PREPARATION

(eg.) Small seeded legumes

Seed coats of these seeds are permeable to TZ and embryos usually will stain without conditioning.

Staining

The prepared seeds should be placed in suitable container (small beakers, petridishes etc.,) and covered with TZ solution. Place the containers in an incubator at dark warm conditions of 40^{o}C.

The staining time varies for different kinds of seeds, different kinds of seeds, different methods of preparation, and different temperatures (< 1 hr to 8 hrs).

When the sample has stained sufficiently the TZ solution should be discarded and the seed sample covered with water immediately. Seed samples can also be kept for 3 days at 10°C for interpretation.

Evaluation of Samples A normal TZ stain appears cherry red.

MONCOTS
NON-VIABLE

1. All structures unstained
2. Shoot largely unstained
3. Scutellar node unstained
4. Major areas of coloeptile unstained
5. Central area of scutellum unstained
6. Insect, mechanical or other injuries causing essential structures non functional.

Dicot seeds Non-viable

1. Embryo completely unstained
2. More than extreme tip of radical unstained
3. More than 1/2 of cotyledon tissue unstained.
4. Deep - seated necrosis at cotyledon and embryonic axis juncture or on radicle
5. Fractured radical.

Advantages of TZ test

1. Quick estimation of viability
2. When the seed is dormant, the TZ test is extremely useful
3. Seeds are not damaged (in dicot) in anlaysis therefore they could be germinated.

Disadvantages of TZ Test

1. It is difficult to distinguish between normal and abnormal seedlings.
2. It does not differentiate between dormant and non- dormant seeds.

Since the TZ test does not involve micro organisms harmful to germinating seedlings are not detected.

GERMINATION EVENTS AND FACTOR AFFECTING THE GERMINATION

Phase I : Imbibition

Imbibition is a precondition for the metabolic process that ultimately lead to completion of the germination process. However, imbibition is a purely physical process which occurs whether the seed is dormant or non-dormant (except physical dormancy), viable or non-viable (Bewley and Black 1994, Mayer and Poljakoff-Mayber, 1982). Hence, dormant or dead seed may imbibe normally without leading to germination. Physically dormant seed will 9ot imbibe unless their seed-coat has been made permeable by pretreatment or natural processes. Even where viable seeds have imbibed, germination may be impeded or delayed by the presence of other types of dormancy or by absence of appropriate germination temperature. Seeds in soil seed banks are often fully imbibed unless physically dormant.

The rate of imbibition and water potential

The rate of imbibition depends on the water potential of .the seed and the soil. Water potential (in physio_ogica literature designated by the Greek letter y) is an expres- sion of the energy status of water. Water 'will tend to flow from a place of high water potential to a place of low potential, and the larger the difference, the higher the flow rate. In common terms if implies that water will flow from a moist media to a dry one, thus from moist soil to a dry seed. The higher the water potential of the soil Le., the damper the soil, the faster the seed will imbibe. Also the dryer the seed, the faster it will imbibe. Therefore, imbibition tends to follow a pattern of an initially high rate which gradually declines as the seed becomes so wet because the water potential is lower in soil than in pure water, and as water moves into the seed, the water potential declines. Water from the soil in the vicinity

will move and replace that taken up by the seed. Rate of water movement in the soil depends on soil structure and moisture content.

Rate of imbibition also depends on size, morphology and internal structure of the seed as well as temperature. Small seeds, seeds that produce mucilage, and seeds with relatively smooth coats tend to be the most efficient in absorbing water owing to their greater contact with soil and their larger surface - area / volume ratio. Imbibition rate also tends to increase with temperature (Bewley and Black 1994). Many dry zone species show a very fast imbibition rate if adequate moisture is available eg'. Many *Acacia* seeds are able to complete imbibition within a few hours when soaked in water once physical dormancy has been broken.

Some seeds show a pattern of imbibition that is different from normal. In legumes, initial imbibition often takes place through the micropyle (unless other parts of the seed- coat have been made permeable example; by scarification). This inflow is often slow, but as the seed takes up water, the entire seed-coat is ruptures and imbibition can now take place through the whole seed-coat. The imbibition curve would here by sigmoid. Very dry seeds sometimes have a slower imbibition rate than more moist ones because water move- ment in dry tissue tends to be physically restricted.

Phase II : Lag phase

Following full imbibition a lag phase of shorter or longer duration normally ap- pears during which water uptake is very low. During this phase metabolic activity com- mences. Both dormant and non-dormant seeds are metabolic active as can be verified *by* ego Dehydrogenase activity, the enzyme forming the basis of tetrazolium viability test.

During this phase the seed mobilizes stored food reserves such as protein and starch and metabolic enzymes become active. As metabolic processes require oxygen, excess moisture with concurrent low oxygen around the seed may easily inhibit processes necessary for germination and the seed may experience delayed germination or in extreme situations it may rot due to anoxia.

Phase III : Cell elongation and mitosis

Following the lag phase seeds enter into a phase of cell elongation and mitosis resulting initially in protrusion of the radicle, later by the appearance of epicotyl, hypocot- yls and cotyledons. Physiologically, seed germination is considered completed on protru- sion of the radicle. In seed testing, germination is considered concluded only once a seed- ling has developed; in the hydrogen peroxide test germinants are evaluated after protrusion of the radicle, but this is considered a viability test, not a germination test.

In dehydrated seed, initial imbibition is associated with leakage of hydrolytes (sug- ars, amino acids, etc.,) from the seed. This leakage is caused by disintegration of cell membranes in dehydrated seeds. In healthy seeds the leakage is of relatively short duration as the membranes rapidly restore themselves.

The respiratory process begins with glucose, a six carbon simple sugar. The first step is glycolysis which involves the phosphorylation of glucose, its conversion to frustose,

the addition of another P group to fructose, the cleavage of the molecule into two 3-C trioses, then the subsequent oxidation and conversion of the triose (glyceraldehyde 3-phos phate) to pyruvic acid through a series of steps. Pyruvic acid is a 3-C organic acid. All of the reactions are catalyzed by specific enzymes and 8 moles of ATP are produced per mole glucose metabolized. Glycolysis or the Embden - Meyerhoff Pathway is shown in the figure.

The Pyruvate produced during glycolysis then enters the Kreb's cycle. The kreb's cycle consumes oxygen, produced CO2, HP and ATP. 12 moles of ATP are produced per mole of acetyl unit metabolized during each cycle.

The continuation of the germination process and early seedling growth is depen- dent on (1) the breakdown of the food reserve into simpler, soluble and translocatable forms and (2) transport of these simpler forms to sites of major metabolic activity (embryonic axis).

The glucose produced by the breakdown of starch is translocated to the scutellum in cereals where it is converted to sucrose, a disaccharide, through enzymic action involv- ing enzymes preexisting or formed in the scutellum. The sugars formed in the scutellum is then translocated to the embryonic axis, where it is converted to glucose. The glucose is metabolized to pyruvic acid via glycolysis and pyruvate enters the Krebs citric acid cycle

where it is further metabolized as previously described. The control mechanisms and

Environmental conditions affecting seed germination.

Germinating seeds are vulnerable, especially during the later phases of germina- tion. Because imbibition is a physical process, seeds may imbibe and dehydrate without damage. As seed enter into the second and third phase with structural changes and cell elongation and divisions, the germination process becomes irreversible: once it has been initiated, it must be completed. conditions differ from one species to another. Several factors interact during germination and for all species a careful balance should be sought between individual factors.

Moisture

Water is a precondition for germination. However, excess water is nearly always damaging since the water tends to replace the soil air and cause compactness, which in turn restricts respiration. Further, excess water promotes development of fungal diseases like damping off. Good soil texture is important for the water - air balance. Because seeds are sensitive to desiccation during the initial germination process, water regulation is especially important during that phase. During germination, only moisture in the immediate vicinity of seedlings is absorbed. Good drainage is necessary to remove excess water.

Aeration

Appropriate aeration is necessary to permit respiration by the roots. Aeration is closely connected to soil structure and moisture conditions.

Light

Seeds with photo dormancy only germinate in light with a high red / far red rela- tion, ego direct sunlight. In practice, light stimulates to overcome dormancy is provided during germination, simply by germinating light - sensitive seeds in light, i.e., only slightly covered. Variation in light requirement may have practical implications. For ego Photodormancy may develop only after a prolonged dark storage. The change from dormant to non-dormant stage of light-sensitive seeds occurs only when seeds are imbibed, so exposure to full sunlight for example during sowing does not provide sufficient stimulus if seeds are dry during sowing. Hence, seeds that are sown deep in the soil may remain photo. dormant, or in extreme cases even develop photo-dormancy because of the relative enrichment of far-red light at greater depth. Germination of seed under the shade of a green canopy may also given insufficient light stimulus for sensitive seeds.

Substrate

The physical structure of the medium in which seeds are germinated is crucial both for germination and early seedling establishment. This is true whether seeds are germinated in a seed-bed and latter transplanted into pots, sown directly in the pots, or sown directly in the field. A good seed bed should provide a balance between moisture and aeration. A loose but fine structure assures a good contact between seed and soil so that water can be supplied continuously, yet provide adequate aeration for respiration by the roots. At the same time, soil structure should allow easy penetration by the roots. Both too loose and too compact soil may influence germination and establishment negatively. Generally, small seeds should have a finer and more compact medium than larger seeds. the soil should be free from clots and the surface should have a texture that will not form a crust (Hartmann *et af.* 1997). Crusting can both be a restriction to aeration and a physical barrier to penetration by the emerging seedling, the latter especially for small seeded species.

A good growth medium for germination is provided by choosing an appropriate substrate and by appropriate soil preparation and management. Obviously, during direct seeding only the latter can be manipulated. For most species a medium loam texture, not too sandy, and not too fine provides the best germination conditions. Incorporation of sand, peat or other material into the available soil type by mixing may be necessary to achieve the desired structure. Sand may be used to improve drainage and aeration. River sand is normally free of toxic salts and thus better than seashore sand. Peat or other material with high organic content improves the water retention capacity.

Seed-bed conditions can be greatly improved by appropriate preparation. Weed and other plant debris should initially be removed, and the soil then worked thoroughly to root depth. This usaully earliest when the soil is dry. Soil from previous years seed-bed may be contaminated by pathogens and may be sterlized by heating (which required that the soil be removed and put back after heating) or by fumigation. The best seed-bed is prepared under slightly damp, but not wet conditions. Once the seed-bed has been worked, and physical compaction such as that caused by walking should be avoided. (Seeber, 1976).

The Optimal planting depth varies with species. Under moist conditions many seeds germinate readily on the surface, the redicle growing into the soil and anchoring the seedling. Hartmann *et al.* (1997) state as a rule of thumb that seeds should be sown at a depth that approximate three to four times their diameter. However, large seeds (> 1.5-2 cm diam.) need only a sowing depth of twice their diameter. For any seed, too deep sowing delays the emergence, and where seeds are sown very deep, emergence may fail altogether. Seeds that need light for germination should obviously only be covered with a shallow layer of soil, but in practice all light-sensitive seeds are relatively small, and are sown shallowly because of their size.

Germination stimulants

Several chemical compounds have a promoting effect on seed germination by stimulating individual metabolic processes during germination. Some compounds may interfere with dormancy and application may partly substitute temperature or light pretreatment. The effect of germination stimulants is thus mostly evident under sub- optimal germination temperatures.

pH

Germination as well as seedling development may be influenced by pH of the ger- mination medium. Lacey and Line (1994) found that pH above 8.5 was detrimental both to total number of germinating seeds and seedling survival.

Orientation

All seeds embryos possess an innate ability to orient themselves and grow accord-

ing to gravity. The phenomenon, known as geotropism, means that the radicle will always grow down into the soil and the shoot up no matter how the micropyle end from which the radicle emerges is oriented. Hence, if the radicle end if facing upwards, the emerging radicle will immediately change direction and grow down. Some energy is, however, wasted during this process, and in some seeds orientation may influence germination. Most seeds are somewhat asymmetrical and are not likely to be oeposited with the radicle end up during natural dispersal. Flat and oblong seeds tend to be deposited in a horizontal position, so that the radicle in most cases needs to change direction only 90° when emerging. Hence, during sowing practices where seeds are broadcast on seed-beds, few seeds are likely to be deposited inversely, and no measures are necessary to assure correct orientation.

Geotropism in germination. The root will tend to grow in the direction of gravity (down) and the shoot opposite no matter how the seed is oriented in the germination bed. If the seed is oriented with the radicle end down and both root and shoot grow straight and if the seed is sown inversely and both radicle and shoot need to change direction after appearance.

IMPORTANT QUESTIONS:

1. What is quick germination test?
2. Explain tetrazolium test for estimation of seed viability.
3. Write down advantage and disadvantage of Tz test for seed viability.
4. What evaluations are done after viability test?
5. Briefly explain different methods for preparation of tetrazolium test.
6. What is germination? Explain the event of germination.
7. Write down the factors affecting germination.
8. Differentiate between epigeal and hypogeal germination.