THE TOPOGRAPHICAL TETRAZOLIUM METHOD FOR DETERMINING THE GERMINATING CAPACITY OF SEEDS

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(WITH TWO FIGURES)

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In 1942 the author published the so-called tetrazolium method for determination of the germinability of seeds (**3**). This method is based upon the reduction in germinability of the seeds due to a gradual dying-off of the embryo (**2**). The topographical spread of necrosis of embryo tissues can be demonstrated by means of 2,3,5-triphenyl-tetrazolium-chloride. This compound is colorless but is reduced (hydrated) into the stable but nondiffusible red formazan by living cells (**3**). Coloration of a cell by tetrazolium is a definite indication of its viability because necrotic cells remain uncolored. By means of extensive experiments conducted over a period of years, the procedure has been perfected to demonstrate which parts of the embryo are at least sufficiently viable to make germination possible (**4**).

The recent publication of R. H. PORTER, MARY DURRELL, and H. J. ROMM (**1**) concerning the topographical tetrazolium method does not, however, present the method correctly in relation to several essential points. In order to correct these errors, the present author submits a short description of his method (**3**).

Use of the tetrazolium method merely involves preparation of the embryo of various species of seeds in such a way that all those parts which are decisive in the estimation of germinability be made visible. In most cereals the anterior face of the embryo must be visible. Since the pericarp is impermeable and opaque in most cereals, the embryo must be removed from the kernel with a lance-like scalpel or dissecting needle. Except in maize, a longitudinal section through the center of the embryo is not adequate because the lateral root primordia remain invisible. Only in oats is the pericarp sufficiently transparent to obviate excision of the embryo. The embryo of maize, however, possesses a simple root primordium, and the lateral root primordia (secondary radicles) in the mesocotyl are uniformly distributed. In this instance a longitudinal section provides the best preparation (**5**). The presoaking of the seeds in water is necessary only to facilitate the removal of the embryo. Initiation of germination is neither necessary nor significant for the test because even non-after-ripened cereals can reduce tetrazolium. According to the method described below, the cross-sectioned grains of oats are placed in tetrazolium solution without pre-soaking. Only in the case of maize is the staining of the scutellum important in relation to germinability. This response of

the scutellum in other species of cereals is of no significance. The tetrazolium procedure, in contrast to the germination tests, practically eliminates experimental errors. Data from two tests of 100 kernels each treated in this manner have been found to provide the same accuracy as direct germination experiments carried out as four tests of 100 grains each. The reason for these results is that the topographic tetrazolium method practically eliminates systematic errors because the tests are independent of personal procedure; in the usual germination tests this individual error amounts to more than half of the total error according to RODEWALD (7). Only the sampling error remains and this will maximally be 50 per cent. higher with 200 kernels than with 400 kernels and therefore will not reach the amount of the total error of germination tests. In fact, statistical processing of several thousand tests has shown that in tetrazolium tests with 200 kernels each, the errors always were lower than in germination tests with 400 kernels.

For small cereals and maize the following procedure is most advantageously employed. A 1 per cent. aqueous solution of 2,3,5-triphenyltetrazolium-chloride is used. This reagent is light-sensitive. In handling it, exposure both to direct sunlight and prolonged exposure to diffused sunlight must be avoided. The solution when preserved in the dark remains unchanged for several months. It should have a pH value between six and seven in order to be properly reactive. According to the writer's extended investigations, a 1 per cent. solution at this pH-value proved most favorable, because it produced best reaction in all cases. For wheat, rye, and barley the desired number of grains is soaked in water from six to 18 hours which is done most conveniently during the night. The embryo is then excised by means of a sharp-bladed dissecting needle as follows: the needle is thrust through the pericarp and testa below the embryo; the scutellum is severed and the embryo removed. The embryo (except the scutellum) must remain undamaged and free from starchy endosperm and pericarp fragments. Embryos extracted in this manner are placed in tetrazolium solution. It is advisable to use small porcelain blocks having a round cavity about 30 mm. in diameter. Special care must be exercised to insure the continuous submersion of embryos in the solution. Prolonged contact with the air must absolutely be avoided.

The reaction reaches completion after about seven to eight hours at room temperature in the dark. The response can then be accurately determined. Only those embryos are germinable in which the plumule and adjacent tissue-bearing root primordia are stained. Coloration of a small portion of these tissues constitutes a positive test. The critical regions are indicated by hatched areas in figure 1. In wheat (fig. 1a) and oats (fig. 1d) (whose embryos possess an epiblast) the borderline between stem and root structures parallels the epiblast. In rye (fig. 1b) this line lies at the base of the upper root primordia; in barley (fig. 1c) this line is recognizable as a distinct constriction between shoot and root structures.

An inexperienced operator may at first consider the excision of embryos both difficult and tedious. The procedure is in reality extremely simple and can be learned in a very short time, often within a few hours. An experienced operator can prepare 200 embryos in about half an hour. Embryos which are decayed and soft cannot be extracted. These are classed as nongerminable.

Oats have a thin and transparent seed coat and hence excision of embryos is not necessary. The grain is evenly cut across by means of small nippers or scissors whereupon the two halves of the grain drop out of the hull. The dehulled halves of the grain which carry the embryo may then be placed in the tetrazolium solution without pre-soaking. It is advisable to place them in small glass dishes about 40 mm. in diameter. After some practice, the determination can be made in about 24 hours at room temperature. In some cases, however, it is advisable to extend the observation to 48 hours because with oats some individual kernels may show a faint coloring, which may cause doubt about the reaction. Criteria of germinability in these cases are the same as for other species of grains, namely coloration of shoot and root primordia without reference to the scutellum. The hatched portion of figure 1d indicates that part of the oat embryo which must at least be stained if the grain is to be classed as germinable.

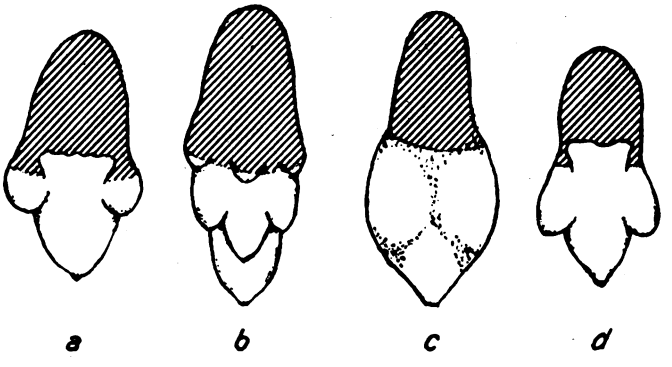

FIG. 1. Excised embryos of cereals, about ×13. a. Wheat; b. Rye; c. Barley; d. Oats. Those embryo parts which at least must show coloration after treatment with tetrazolium in order for the kernel to be germinable are shown by hatch marks.

In the case of maize (5) the grains to be tested are soaked in water for several hours, preferably over night. Then by means of a sharp scalpel, the kernels are cut longitudinally so that the embryo is bisected medianally throughout its entire length. One half of each grain is put into a 9 cm. petri dish and covered with tetrazolium solution until the grains are barely submerged therein. Contact of the tissue surfaces with the air must be avoided under all circumstances. The determination will be possible after three to four hours. Those grains are germinable in which the embryo is stained either completely or at least in the region of the shoot, including initials of secondary radicles and the scutellum. Coloration of

the corn scutellum, in contrast to other cereals, is significant and at least half of its area (the medial zone) should show coloration (fig. 2). The tissue connecting the germ and scutellum should be stained in its entirety. The minimum area requisite as a test of germinability is shown as the hatched portion of figure 2.

In especially urgent cases involving need for haste one may utilize tepid water for pre-soaking, and seed-parts submersed in the tetrazolium solution can be placed in an incubator at 30° C. In this manner the test may be shortened to a single day.

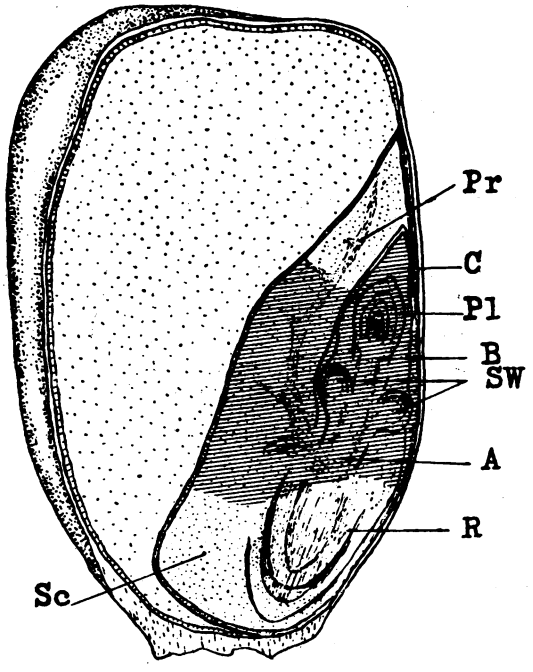

FIG. 2. Longitudinal median section through a kernel of corn. Sc, scutellum; Pr, procambial strand; C, coleoptile; Pl, plumule; B, base of plumule; A, transition from plumule to radicle; R. radicle; SW, initials of secondary radicles. That portion of the embryo which at least must show coloration after treatment with tetrazolium in order for the kernel to be germinable is shaded by hatch marks. About ×8.

The staining must be completed within 24 hours at the most. During longer periods, microorganisms may appear and obscure the reaction. This difficulty may, however, be overcome by transferring the seeds or embryos into a 1/1000 solution of mercuric chloride. In this case the test may be read at a later time.

Bacteria which multiply rapidly, especially in oat and maize tests even within the normal reaction time in tetrazolium, may cloud the picture in case of severe infection of the medium. Living bacteria, which are also stained by tetrazolium, may erroneously simulate staining of the cereal embryos or of the cut surface of the maize kernels. In such cases the

tetrazolium solution itself is stained red and this abnormal discoloration of the reagent enjoins caution. In such instances seed parts appear improperly stained, differing distinctly from normal coloration. Maize endosperm, for instance may be covered with minute red spots. This colored deposit can, however, be removed by gentle abrasion of the surface with a dissecting needle or scalpel and the correct test of underlying tissue can then be accurately determined.

In comparison with direct germination tests, the above tetrazolium procedure has the following advantages:

1. No great amount of space or large and complex apparatus is required. Tests can be conducted in a limited space with simple instruments such as porcelain blocks, petri dishes and small glass dishes, dissecting needles, scalpels, scissors or nippers. The tests themselves are simple and conclusive.

2. Execution is rapid even for large scale tests, small cereals requiring no more than 48 hours, and maize no more than one day at the most. In especially urgent cases the time may be considerably reduced by presoaking in tepid water and incubation at 30° C as described.

3. The method provides reliable and exact results. Even in the case of impediments to germination as in freshly harvested non-after-ripened cereals which are not immediately germinable but which possess inherent germinability of the grain (a condition which gave rise to the term "germinating potency" or "Keimpotenz" (**6**) in 1918), the eventual germination can be determined with certainty.

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LITERATURE CITED

1. PORTER, R. H., DURELL, MARY, and ROMM, H. J. The use of 2,3,5-triphenyl-tetrazolium-chloride as a measure of seed germinability. Plant Physiol. **22**: 149–159. 1947.
2. LAKON, GEORG. Das Schwinden der Keimfähigkeit der Samen, insbesondere der Getreidefrüchte. Ber. Deutsch. Bot. Ges. **57**: 191–203. 1939.
3. ————. Topographischer Nachweis der Keimfähigkeit der Getreidefrüchte durch Tetrazoliumsalze. Ber. Deutsch. Bot. Ges. **60**: 299–305. 1942.
4. ————. Die topographische Selenmethode, ein neues Verfahren zur Feststellung der Keimfähigkeit der Getreidefrüchte ohne Keimversuch. Proc. Intern. Seed Testing Assoc. **12**: 1–8. 1940.
5. ————. Topographischer Nachweis der Keimfähigkeit von Mais durch Tetrazoliumsalze. Ber. Deutsch. Bot. Ges. **60**: 434–444. 1942.

6. ————————. Ueber Keimpotenz und labile Keimtendenz bei Pflanzensamen, insbesondere bei Getreidefrüchten. Festschrift z. 100 jähr. Bestehen der Landw. Hochschule Hohenheim. 70–83. 1918.

7. RODEWALD, H. Untersuchungen über die Fehler der Samenprüfungen. Arbeit. Deutsch. Landw.-Gesellsch. Heft. 101. 1904.